International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/31, 15/63, 15/67, 15/70, 15/74, 15/80, 15/82, 15/85, 15/11, 9/90, 1/19, 1/21, 5/10, C07K 14/37, A01K 67/027

A1

(11) International Publication Number: WO 00/32785

(43) International Publication Date: 8 June 2000 (08.06.00)

(21) International Application Number: PCT/IT99/00391

(22) International Filing Date: 29 November 1999 (29.11.99)

(30) Priority Data:
RM98A000730 30 November 1998 (30.11.98) IT

(71) Applicant *(for all designated States except US)*: UNIVERSITA' DEGLI STUDI DI ROMA "LA SAPIENZA" [IT/IT]; Piazzale Aldo Moro, 5, I–00185 Roma (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MACINO, Giuseppe [IT/IT]; Policlinico Umberto I, Università Degli Studi Di Roma "La Sapienza", Dipartimento Biotecnologie Cellulari ed Ematologia, Viale Regina Elena, 324, I–00161 Roma (IT). COGONI, Carlo [IT/IT]; Policlinico Umberto I, Università Degli Studi Di Roma "La Sapienza", Dipartimento Biotecnologie Cellulari ed Ematologia, Viale Regina Elena, 324, I–00161 Roma (IT).

(74) Agents: BANCHETTI, Marina et al.; Ing. Barzanò & Zanardo Roma S.p.A., Via Piemonte, 26, I–00187 Roma (IT).

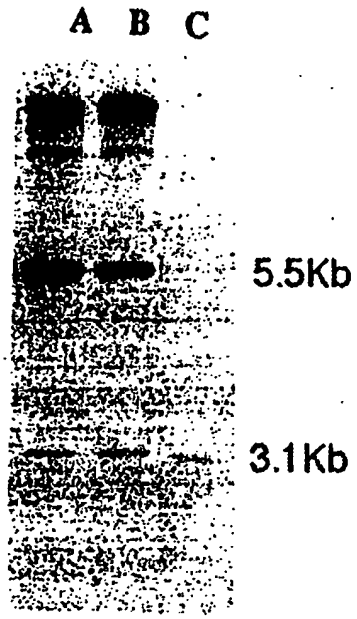
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ISOLATION AND CHARACTERIZATION OF A *N. CRASSA* SILENCING GENE AND USES THEREOF

(57) Abstract

A nucleotide sequence encoding for a protein characterized in that it has a silencing activity and comprises a *recQ* helicase domain is disclosed; furthermore expression vectors suitable for the expression of said sequence in bacteria, plants, animals and fungi are disclosed; the invention refers also to organisms transformed by such vectors.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

ISOLATION AND CHARACTERIZATION OF A *N. CRASSA* SILENCING GENE AND USES THEREOF

The present invention relates to the isolation and characterization of a *Neurospora crassa* gene encoding for an essential activity in the co-suppression process and to uses and applications thereof in vegetal, animal and fungine fields.

The production of transgenic organisms is of large utility both in basic and applied biological research. The transgenic DNA is usually integrated in the genome and transferred as a Mendelian character. However, in various instances, the transgene introduction induces gene silencing phenomena (Flavell, R.B. 1994), i.e. the repression of the expression of the transgene itself and/or of one or more endogenous homologous genes.

The gene silencing can act at two levels: transcriptional (trans-inactivation) where transgenes contain sequences homologous to the silenced gene promoter (Vaucheret, 1993); and post-transcriptional (co-suppression) which requires homologies between coding regions (Flavell, 1994; Stam et al., 1997; Baulcombe, 1996).

Generally the silencing induced by a transgene requires an almost complete sequence homology (from 70% to 100%) between transgene and silenced gene sequences (Elkind, 1990).

In the *Neurospora crassa* filamentous fungus, during the vegetative phase, the presence of transgenes induces a post-transcriptional gene silencing phenomenon, named "quelling" (Cogoni et al., 1996).

By using the *al-1* gene (albino 1) (Schmidhauser et al., 1990) as silencing visual marker, many features of the phenomenon have been discovered (Cogoni et al., 1996). Particularly the *al-1* gene "quelling" in *Neurospora* is characterized in that: 1) the gene silencing is reversible further to the loss of transgene copies; 2) the reduction of mRNA basal level results from a post-transcriptional effect; 3) transgenes containing at least a region of 132 base pairs which is identical to the region encoding for the target gene are sufficient to induce the "quelling"; 4) the duplication of promoter sequences is ineffective to induce the silencing; 5) the "quelling" exhibits a dominant behavior in eterocarions containing both transgenic and untransformed nuclei, indicating the involvement of a molecule which is acts "in trans" among the nuclei; 6) the expression of an aberrant RNA transcribed by the transgenic locus is strictly correlated to silencing, suggesting that the "quelling" can be induced and/or mediated by a transgenic RNA molecule.

Therefore homologies between *Neurospora* silencing and plant co-suppression can be pointed out. The gene silencing in *Neurospora* is reversible, as result of transgenic copies instability during mitotic phase; in plants also the co-suppression reversion is associated with the reduction of transgene copy number, resulting from intra-chromosomal recombination during mitosis or meiosis (Mittelstein Scheid et al., 1994; Stam et al., 1998). Thus both in plants and in *Neurospora* the transgene presence is required to maintain the silencing. As in *Neurospora,* a decrease of the mRNA basal level of the silenced gene results from a post-transcriptional

mechanism (Dehio and Schell 1994; van Blokand et al., 1994; de Carvalho et al., 1995). Furthermore to induce the "quelling", transgenes must contain a portion of the silencing target gene coding sequence, being the promoter region ineffective. In plants coding regions with no promoter sequences can induce silencing (van Blokand et al., 1994) and, as in the "quelling", promoters or functionally active gene products are not required for the co-suppression.

One of the similarities between "quelling" and co-suppression in plants is that both mechanisms are mediated by diffusion factors. In *Neurospora* eterokaryotic strains, nuclei wherein the *albino-1* gene is silenced are able to induce the *al-1* gene silencing of the other not transformed nuclei, all sharing the same cytoplasmic environment (Cogoni et al., 1996). In plants the presence of a diffusion factor results from the fact that the co-suppression is effective in inhibiting the replication of Tobacco Etch Virus (TEV), a RNA virus with an exclusively cytoplasmic cycle. The occurrence of highly diffusible factors, which are effective to mediate the co-suppression, has been demonstrated using the grafting technique in tobacco (Palaqui et al., 1997), showing that silenced tobacco plants are able to transfer the silencing to non-silenced plants through grafting.

The fact that "quelling" and co-suppression share all these features suggests that mechanisms involved in post-transcriptional gene silencing in plants and in fungi can be evolved by an ancestral common mechanism.

Recently gene inactivation phenomena resulting from transgene introduction have been disclosed in animals. In *Drosophila melanogaster* the location of a transgene close

to heterochromatic centers results in a variegate expression (Wallrath and Elgin, 1995; Pirrotta, V., 1997). Similar expression profiles have been observed when the reference transgene is within tandem arrayed transposons, indicating that tandem repeats are effective to induce the chromatin condensation. (Dorer and Henikoff, 1994). Again in *Drosophila* Pal-Bhadra et al. (1997) have observed that the transgene introduction can lead to gene inactivation phenomena, similar to the co-suppression.

Gene silencing phenomena resulting from transegene sequence repeats have been disclosed recently in mammalians.

Garrick et al. (1998) produced mouse transgenic lines wherein 100 transgenic copies are present only in a locus and are directly tandem arrayed. The transgene expression has been disclosed to be inversely proportional to the number of occurring copies, indicating that silencing phenomena dependent on repeat copies are present also in mammalians.

Therefore the identification of *Neurospora* genes which are involved in the silencing is the first step to modulate the same process in plants, animals and fungi. The silencing modulation is of great relevance when transgenic organisms able to express the desired phenotype are produced.

The authors of the present invention have already isolated *Neurospora crassa* strains having mutations regarding essential functions for gene silencing mechanism (Cogoni and Macino, 1997); 15 independent isolated mutants define three complementation groups, thus identifying the *qde-1, qde-2 and qde-3* genes (*qde*

stands for "quelling"-deficient), whose products are essential to the silencing machinery. *qde* genes are essential to the *Neurospora* silencing, as suggested by the fact that silencing of three independent genes (*al-1*, *al-2* and *qa-2*) is impaired by *qde* mutations (Cogoni and Macino, 1997).

The authors of the invention have identified and cloned now one out of *Neurospora qde* genes, thus identifying one of required factors for silencing. By considering the similarity between "quelling" and co-suppression, genes orthologous to the isolated gene are involved in co-suppression and more generally in gene silencing in other organisms, like plants, fungi and animals.

The present invention can be applied with reference to two general scope: 1) silencing potentiation as a tool for inactivating more effectively and durably a desired gene, and 2) silencing suppression to obtain a better expression of the introduced transgenes.

As to the silencing potentiation, the over-expression of one or more genes controlling the phenomenon can lead to higher efficiency and/or stability thereof. Therefore the introduction of *qde-3* gene or of homologous genes thereof in microorganisms can constitute a tool to repress more effectively gene functions. Particularly this approach is specially useful in plants wherein the co-suppression is usually used for the "knock-out" of gene functions. In plants again the gene silencing potentiation can be used to obtain lines resistant to pathogen virus, by introducing transgenes encoding for viral sequences, in order to achieve the

expression inhibition of the virus itself (Flavell et al., 1994).

Analogous applications are suitable for animals, wherein some indications suggest that silencing can inhibit the suitable expression of introduced transgenes (Garrick et al., 1998).

On the contrary, there are instances wherein it is desirable not to have or to reduce the gene silencing, i.e. where a transgene is to be over-expressed. It is known that the co-suppression is strictly correlated both with the presence of an high copy number of the transgene, and with a transgene high expression. This correlation can hamper the production of transgenic organisms which express a transgene at high levels, because more high is the expression and/or the copy number, more probable is to evoke silencing responses. As above mentioned, analogous mechanisms of gene inactivation, dependent on a high copy number, have been disclosed in animals. In these circumstances plant or animal lines, totally or partially ineffective for silencing, constitute an ideal recipient wherein the desired gene can be over-expressed. The invention can be applied within this scope using different approaches:

A) Identification and production of mutant lines in genes homologous to *qde-3* gene, in plants, animals and fungi.

The knowledge of *Neurospora qde-3* gene, essential for silencing mechanism, can allow the isolation of mutant lines in other organisms, mutated in genes homologous to *qde-3*. For example by means of amplifications using degenerated primers, designed from the most conserved regions of *qde-3* gene, mutant lines in

homologous genes can be identified, by analysis of insertion mutant gene banks, already available for many plant species. Both in fungi and animals such mutants can be obtained, following the identification of the homologous gene, by means of "gene disruption" techniques using homologous recombination.

B) Reduction of *qde-3* gene expression

Other strategies for the production of silencing-deficient lines comprise the use of *Neurospora qde-3* gene or homologous genes thereof. *qde-3* or homologous genes can be introduced into suitable expression vectors to express them in an anti-sense orientation in order to inhibit the expression of resident endogenous genes. Alternatively portions of *qde-3* or of homologous genes can be over-expressed, in order to obtain a negative dominant effect and thus blocking the function of qde-3 endogenous genes.

The authors of the present invention have cloned and characterised the *Neurospora crassa qde-3 gene*. The sequence analysis showed that *qde-3* gene belongs to a highly conserved gene family, from *E. coli* to humans, named *recQ*. Genes belonging to this family encode for DNA helicase, as demonstrated by *in vitro* assays (Gray et al., 1997). The recQ helicase family is involved in recombinant processes. Mutations of these genes produce iper-recombinant phenotypes as, for example, the *S. cerevisiae* Sgs-1 gene involved both in meiotic and mitotic recombination.

The authors of the invention for the first time have demonstrated that a gene encoding for a recQ DNA-helicase is involved in gene silencing induced by transgenes. Therefore for the first time it is disclosed

that a gene belonging to the *recQ* family, other than acts during recombination, is also an essential component of the inactivation of repeat sequences.

Therefore it is an object of the invention a nucleotide sequence encoding for a protein characterized in having a silencing activity and comprising a recQ helicase domain, wherein the domain is at least 30% homologous with the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1. More preferably said homology is of at least of 60%. Most preferably the recQ helicase domain comprises the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1. According to a particular embodiment the nucleotide sequence encodes for a protein having the amino acid sequence of SEQ ID No. 1, or functional portions thereof. Even more preferably the nucleotide sequence of the invention is the sequence of SEQ ID No. 1 or its complementary sequence.

A further object of the invention is an expression vector comprising, under the control of a promoter that is expressed in bacteria, the nucleotide sequence of the invention. Those skilled in the art will appreciate that any plasmid suitable for a correct and effective expression of the protein of the invention in bacteria can be used and is within the scope of the invention.

A further object of the invention is an expression vector comprising, under the control of a promoter which is expressed in plants or in specific plant organs, the nucleotide sequence of the invention, both in a sense and anti-sense orientation. Those skilled in the art will appreciate that any plasmid suitable for a correct and effective expression of the protein of the invention in

plants or in specific plant organs can be used and is within the scope of the invention.

A further object of the invention is an expression vector comprising, under the control of a promoter which is expressed in fungi or in portions thereof, the nucleotide sequence of the invention, both in a sense and anti-sense orientation. Those skilled in the art will appreciate that any plasmid suitable for a correct and effective expression of the protein of the invention in fungi or in portions thereof can be used and is within the scope of the invention.

A further object of the invention is an expression vector comprising, under the control of a promoter that is expressed in animals, the nucleotide sequence of the invention both in a sense and anti-sense orientation. Those skilled in the art will appreciate that any plasmid suitable for a correct and effective expression of the protein of the invention in animals can be used and is within the scope of the invention.

A further object of the invention is a prokaryotic organism transformed by using the expression vector active in bacteria of the invention.

A further object of the invention is a plant or a specific plant organ transformed by using the expression vector active in plants of the invention.

A further object of the invention is a plant mutated at the nucleotide sequence of the invention and having a reduced or inhibited silencing activity.

A further object of the invention is a fungus transformed with the expression vector of the invention active in fungi.

A further object of the invention is a fungus mutated at the nucleotide sequence of the invention and having a reduced or inhibited silencing activity.

A further object of the invention is a non-human animal transformed with the expression vector of the invention active in animals.

A further object of the invention is a non-human animal mutated at the nucleotide sequence of the invention and having a reduced or inhibited silencing activity.

A further object of the invention refers to a protein characterized in having a silencing activity and in comprising a recQ helicase domain, wherein the domain is at least 30% homologous to the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1. Preferably the recQ helicase domain is at least 40% homologous with the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1. More preferably the recQ helicase domain is at least 60% homologous with the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1. Most preferably the recQ helicase domain comprises the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1. According to a particular embodiment the protein comprises the amino acid sequence of SEQ ID. No.1 or functional portions thereof.

It is within the scope of the invention the use of the nucleotide sequence of the invention to modulate gene silencing in plants, animals and fungi.

It is within the scope of the invention the use of the nucleotide sequence of the invention to potentiate the antiviral-response in a plant.

The present invention now will be disclosed by way of non limiting examples with reference to the following figures:

Figure 1: Southern blot analysis of genomic DNA extracted from (A): untransformed wild type strain, (B): 6xw recipient strain and (C): untransformed wild type strain, SmaI and HindIII digested, blotted and *al-1* gene probe hybridized. The 3.1-Kb band corresponds to the endogenous *al-1* gene, while the 5.5-Kb band corresponds to tandem arrayed *al-1* transgenes. The larger band represents undigested methylated DNA.

Figure 2: Linear map of the pMXY2 plasmid. Plasmid genes are shown as box. bmI: beta-tubulin allele which is responsible for benilate resistance; Amp: ampicillin resistance; qa-2 P: qa-2 gene promoter; TrpC T: trpC gene terminator. SphI and BglII are restriction sites used for the plasmid recovery from the 627 mutant chromosomal DNA.

Figure 3: Schematic representation of pQD6 and pQ35 plasmids. Restriction sites (BglII for pQD6 and SphI for pQ35) used for the recovery of the chromosomal DNA of the 627 strain are reported. Chromosomal sequences, flanking the integration site, are represented as segments. Restriction sites used to isolate DNA fragments used for probing the gene library are also represented.

Figure 4: Nucleotide sequence of the 6.9-Kb fragment containing the *qde-3* gene and flanking sequences. The amino acid sequence is shown above the nucleotide sequence. The bold sequences represent two introns of 98 and 68 nt. In these regions the underlined nucleotides identify consensus sequences of the donor site, the acceptor site and the internal sequence or lariat. It is also represented the pMXY2 plasmid

insertion site, in the 627 mutant, used for insertional mutagenesis. The portion encoding for the helicase domain is underlined.

Figure 5: Nucleotide sequence (SEQ ID No. 1) of the encoding portion reported in Figure 4 and deduced amino acid sequence. Amino acids from 897 to 1330, which define the recQ DNA-helicase domain, are underlined.

Figure 6: Multiple alignment, at the conserved domains, among *qde-3* and other proteins belonging to *recQ* family. arab *recQ*: A. *thaliana* isologous; *E. coli recQ; S. pombe hus-2; S. cerevisiae sgs-1;* human wrn: Werner syndrome; human blm: Bloom syndrome. Identical amino acids are shown in bold.

MATERIALS AND METHODS

E. coli strains

E. coli strain HB101 (F^-, hsdS20(rb^-, mb^-), supE44, recA13, ara14, proA2, rspL20(str^r), xyl-5) was used for cloning.

Neurospora crassa strains and growing conditions

Neurospora crassa following strains, supplied by Fungal Genetic Stock Center (FGSC, Dpt. Of Microbiology, University of Kansas Medical Ctr. Kansas City, KA) were used:

- Wild type (FGSC 987);
- qa-2/aro9 (FGSC 3957A), (FGSC 3958a).

The 6XW strain (Cogoni et al., 1996) was obtained upon transformation of the FGCS 3958a strain with pX16 (Cogoni et al., 1996). This plasmid contains the *qa-2* gene used as selective marker and the *al-1* coding sequence.

The mutated strains M7, M20 (*qde-1*); M10, M11 (*qde-2*); M17, M18 (*qde-3*) are described in Cogoni and Macino, 1997.

The *qde* mutants were obtained by UV mutagenesis. As recipient the transforming strain (6xw) silenced at the *albino-1* gene was used. *qde* mutants were selected for their ability to recover a wild type unsilenced phenotype and then classified in three different complementation groups. By analyzing the *al-2* gene quelling frequency all of *qde* used mutants are defective for the general silencing mechanism.

Complementation assays with not forced heterocaryons were carried out according to Davis and DeSerres, 1970.

Plasmids and libraries

The plasmid pMXY2, disclosed in Campbell et al., used for insertional mutagenesis was obtained from FGSC. The plasmid contains the *Bml* gene (allele responsible of the benilate drug resistance), that was used as selective marker after transformation. The genomic DNA containing the *qde-2* gene was isolated from a *N. Crassa* gene library in cosmids. (Cabibbo et al., 1991).

N. crassa transformation

Spheroplasts were prepared according to the Akins and Lambowitz (1985) protocol.

Southern Blot Analysis

Chromosomal DNA was prepared as disclosed by Irelan et al., 1993. 5 μg of genomic DNA were digested and blotted as reported in Maniatis et al.

DNA probes were: a) as to the *al-1* gene the probe is represented by a XbaI-ClaI restriction fragment of

pX16 (Cogoni et al., 1996); b) as to the *BmI* gene the probe is represented by the 2.6Kb SalI fragment of pMXY2.

Northern Blot Analysis

N. crassa total RNA was extracted according to the protocol described by Cogoni et al., 1996. The mycelium was grown for two days at 30°C, then powdered in liquid nitrogen before RNA extraction. For Northern analysis 10 µg of RNA were formaldehyde denatured, electrophoresed on a 1% agarose, 7% formaldehyde gel, and blotted over Hybond N (Amersham) membranes. Hybridization was carried out in 50% formamide in the presence of ^{32}P labeled DNA probe $1.5x10^6$ cpm/ml.

RESULTS

Isolation of silencing mutant by insertional mutagenesis

Neurospora strain (6XW) wherein the *albino-1* resident gene was steadily silenced was UV mutagenised, and *qde* ("quelling" deficient) mutants were isolated (Cogoni and Mancino 1997). The 6XW strain shows an albino phenotype due to the lack of carotenoid biosynthesis, as results by the silencing of the albino 1 gene expression (Schmidhauser et al., 1990). A mutation interfering with the silencing machinery is easily detectable by producing a wild type phenotype (bright orange) of the carotenoid biosynthesis. By means of complementation assays it was possible to establish that *qde* mutants belong to three complementation groups, indicating the presence of three genetic loci involved in the *Neurospora* silencing mechanism. In order to isolate the *qde* genes an insertional mutagenesis was carried out with the 6XW strain, previously used for UV mutagenesis. The insertional mutagenesis was carried out by transforming the 6XW strain with a plasmid, taking advantage of the

fact that, after the transformation, plasmids are randomly inserted in the *Neurospora crassa* genome. The mutagenesis was carried out transforming the 6XW silenced strain with pMXY2 (see Materials and Methods) which contains the benilate resistance as selective marker. Transformed strains able to grow in the presence of benilate containing medium and showing a wild type phenotype for the carotenoid biosynthesis were selected. Out of 50.000 isolated independent transformed strains, a benilate resistant strain (627) was isolated, which showed the bright orange phenotype expected for a *qde* gene mutation. In order to verify that the silencing release was effectively due to a *qde* gene mutation and not to the loss of *al-1*, the genomic DNA of the strain 627 was extracted and digested with SmaI and HindIII restriction enzymes. After blotting, DNA was hybridized with a probe corresponding to the coding sequence *of al-1*. The SmaI site is present only once in the *al-1* transgene containing plasmid and the digestion by using said enzyme produces a 5.5Kb fragment corresponding to tandem arrayed *al-1* transgenes, while a 3.1Kb fragment is expected from the resident *al-1* locus. Figure 1 shows that the number of *al-1* transgenic copies present in the 627 strain is comparable to that present in the silenced 6XW strain.

The 627 strain includes a mutated *qde3* gene

The 627 strain was assayed in a heterokaryon assay with a wild type strain and with M7, M20 (*qde-1*) M10, M11 (*qde-2*) mutants (Cogoni and Macino, 1997). As shown in Table 1 the *al-1* gene silencing is restored producing an albino phenotype in all of heterocaryons but M17 and M18.

This behavior is consistent with the presence of a *qde-3* gene recessive mutation in the 627 strain.

Table 1

Reciprocal heterokaryons among 627 mutant and previously characterized *qde* mutants.

	627	M7	M20	M10	M11	M17	M18
627	WT	AL	AL	AL	AL	WT	WT
M7		WT	WT	AL	AL	AL	AL
M20			WT	AL	AL	AL	AL
M10				WT	WT	AL	AL
M11					WT	AL	AL
M17						WT	WT
M18							WT

WT = heterokaryon with a wild type phenotype for carotenoid;

AL = heterokaryon with an albino phenotype wherein the al-1 gene silencing is restored.

Recovery of sequences flanking the pMXY2 plasmid integration site

In order to recover sequences flanking the integration site or sites the following methodology was carried out. The 627 strain genomic DNA was restricted with SphI and BglII enzymes. As shown in the map of Figure 2 the enzymes digest respectively upstream and downstream to the region containing both the ampicillin resistance gene and the origin of replication present in pMXY2. Subsequently the genomic DNA was ligated and the product used to transform *E. coli* cells. The screening was performed in an ampicillin-containing medium. pQD6 and pQ35 plasmids were recovered from BglII and SphI

restricted chromosomal DNA, respectively (see Figure 3). Two DNA fragments containing sequences flanking the integration site were isolated by using, respectively, BglII and SalI enzymes for pQD6, and SphI and HindIII enzymes for pQ35 (Figure 3).

Isolation of genomic clones, their subcloning and complementation of the *qde-3* mutant

The two fragments from pQD6 and pQ35 plasmids were used to probe a *Neurospora crassa* genomic library in cosmids. Cosmids 6E8 and 54D7, both containing about 30 Kb genomic DNA inserts, were isolated. Both the probes recognize the same cosmids, thus indicating that the two flanking sequences are contiguous. Cosmids 6E8 and 54D7 were used in transformation experiments with M17 and M18 mutants. Both of cosmids are able to restore the *al*-1 gene silencing in the two mutants, determining an albino phenotype. Furthermore the introduction of same cosmids into the M10 (*qde-2*) or the M20 mutant (*qde-1*) is not effective to restore the silencing.

The 6E8 cosmid was used to subclone a 9 Kb SphI-SphI fragment. This subclone was used for transformation experiments and resulted to be able to complement the *qde-3* phenotype, indicating that a *qde-3* functional gene is present in this plasmid.

Isolation and sequence of the qde-3 cDNA

The SphI-SphI region was sequenced, like the corresponding cDNA, by using RT-PCR. The latter sequence was used to deduce the qde-3 amino acid sequence and map the introns therein. The qde-3 gene encodes for a 1900 aa. putative protein (200 KDa). The genomic clone contains two introns of 98 nt. and 68 nt., respectively. Intron acceptor and donor sequences were identified and

correspond to described consensus sequences (Figure 4). Furthermore the pMXY2 plasmid insertion site within the gene in the 627 transforming strain is indicated. The insertion site was deduced by analysis of pQD6 and pQ35 plasmid sequences.

The cDNA sequence is shown in Figure 5 (SEQ ID No. 1), wherein the helicase domain containing 434 amino acids from 897 aa to 1330 aa is underlined.

The *qde-3* gene is belonging to recQ helicase DNA family

The 1900 aa sequence was used to search in database of amino acid sequences, by using the BLASTP algorithm. Significant homologies were identified with 6 genes belonging to the *reQ* family, belonging to the helicase group containing the DEAH consensus sequence. Figure 6 shows the homologous region sequence alignment of helicase domains, as defined in Figure 5, among *qde-3* and genes belonging to *recQ* helicase family. *qde-3* shows the highest homology with *hus-2* (55% amino acid identity) and the lowest homology with *Wrn* (40% identity).

Plant expression vector

The *qde-3* gene was inserted, in a sense orientation, into a vector containing a plant expression "cassette", including the 35S promoter and the PI-II "terminator" sequences. The vector also includes the *Streptomyces hygroscopicus bar* gene, which confers the phosphinotricine herbicide resistance to transformed plants. In an analogous vector, *qde-3* was inserted in an anti-sense orientation with respect to the 35S promoter.

The obtained vectors can be utilized to over-express the *qde-3* gene in plants, or to repress the gene expression of resident genes, which are homologous to *qde-3*, respectively.

Fungus expression vector

The *qde-3* gene was inserted in a vector containing a fungal specific expression "cassette", comprising the *A. nidulans trpC* gene promoter and terminator, both in a sense and an anti-sense orientation. In addition the vector contains the bacterial *hph* gene, which confers the hygromicine drug resistance. The sense plasmid can be used to over express the *qde-3* gene, whereas the anti-sense plasmid is used to repress the expression of *qde-3* homologous genes in various fungine species.

Mammalian expression vector

The *qde-3* gene was inserted in a vector containing a mammalian specific expression "cassette", including the cytomegalovirus (CMV) promoter and SV40 termination and polyadenylation sequences both in a sense and anti-sense orientation. The vector includes also the neomicine phototransferase gene, as marker for mammalian cell selection. The sense plasmid can be used to over express the *qde-3* gene, whereas the anti-sense plasmid can be used to repress the expression of *qde-3* homologous genes in various mammalian species.

Bibliography

- Akins, R.A. and Lambowitz A.M. (1985) Mol. Cell. Biol. 5:2272-2278
- Baulcombe, D.C. (1996) Plant Mol. Biol. 32, 79-88.
- Cogoni, C. et al. (1996) EMBO J. 15, 3153-3163
- Cogoni, C. and Macino, G. (1997) Proc. Natl. Acad. Sci. U.S.A. 94: 10223-10238.
- Cabibbo, A. et al. (1991) Fungal Genetic Newsl., 38: 68-70.
- Davis, R.H. and De Serres, F.J. (1970) methods Enzymol. 17: 79-143.

- de Carvalho Niebel, F. et al. (1995), Plant Cell : 347-358.
- Dehio, C., and Schell, J. (1994). Proc. Natl. Acad. Sci. U.S.A. 91: 5538-5542.
- Dorer, D.R. and Henikoff, S. (1994). Cell, 993-1002.
- Elkind, Y. Et al. (19990) Proc. Natl. Acad. Sci. U.S.A. 87: 9057-9061.
- Ellis, N.A. et al. (1995) Cell 83:655-666.
- Flavell, R.B. (1994) Proc. Natl. Acad. Sci. U.S.A. 91: 3490-3496.
- Garrick D., et al. (1998) Nature Genetics 18, 56-59.
- Gray, M.D. et al. (1997) Nature Genetics 17, 100-103.
- Irelan, J. et al. (1993) Fungal Genetics Newsl. 40: 24.
- Lindbo, J.A. et al. (1993) Plant Cell 5 : 1749-1759.
- Maniatis, S.T. et al. (1982) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- Mittelstein Scheid, O. Et al. (1994) Mol. Gen. Genet. 244: 325-330.
- Orbach, M.J. et al. (1986) Mol. Cell. Biol., 6, 2452-2461.
- Pal-Bhadra, M., et al., (1997). Cell 90, 479-490.
- Palauqui, J.C. et al., (1997) EMBO J. 16: 4738-4745.
- Pirrotta, V. (1997). TIG 13, 314-318.
- Schmidhauser, T.J. et al., Mol. Cell. Biol. 10: 5064-5070
- Stam, M. et al. (1997) Annals of Botany 79:3-12
- Stewart, E. et al., (1997) EMBO J. 10:2682-2692
- van Blokland, R. et al. (1994), Plant, 6, 861-887.

- Vaucheret, H. (1993), C.R. Acad. Sci. Paris, Sciences de la vie/Life sciences 316, 1471-1483.
- Wallrath, L.L. and Elgin, S.C.R. (1995). Genes & Development 9, 1263-1277.
- Watt, P.M. et al. (1995). Cell, 81: 253-260.
- Watt, P.M. et al., (1996). Genetics 144: 935-945.
- Yu, C.E. et al. (1996). Science 272: 258-262.

Claims

1. Nucleotide sequence encoding for a protein characterized in having a silencing activity and in comprising a *recQ* helicase domain, wherein the domain is at least 30% homologous with the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

2. Nucleotide sequence encoding for a protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 1, wherein the domain is at least 40% homologous with the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

3. Nucleotide sequence encoding for a protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 2, wherein the domain is at least 60% homologous with the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

4. Nucleotide sequence encoding for a protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 3, wherein the recQ helicase domain is the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

5. Nucleotide sequence encoding for a protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 4, wherein said nucleotide sequence encodes for a protein having the amino acid sequence of SEQ ID No. 1, or functional portions thereof.

6. Nucleotide sequence encoding for a protein characterized in having a silencing activity and

comprising a *recQ* helicase domain according to claim 5, wherein said nucleotide sequence is the sequence of SEQ ID No. 1 or its complementary sequence.

7. Expression vector comprising, under the control of a promoter that is expressed in bacteria, the nucleotide sequence according to any one of claims 1-6.

8. Expression vector comprising, under the control of a promoter that is expressed in plants or in specific plant organs, the nucleotide sequence according to any one of claims 1-6, both in a sense and anti-sense orientation.

9. Expression vector comprising, under the control of a promoter that is expressed in fungi, the nucleotide sequence according to any one of claims 1-6 both in a sense and anti-sense orientation.

10. Expression vector comprising, under the control of a promoter that is expressed in animals, the nucleotide sequence according to any one of claims 1-6 both in a sense and anti-sense orientation.

11. Prokaryotic organism transformed by using the expression vector active in bacteria according to claim 7.

12. Plants or a specific plant organ transformed by using the expression vector active in plants according to claim 8.

13. Plant mutated at the nucleotide sequence according to any one of claims 1-6 having a reduced or inhibited silencing activity.

14. Fungus transformed by using the expression vector active in fungi according to claim 9.

15. Fungus mutated at the nucleotide sequence according to any one of claims 1-6 having a reduced or inhibited silencing activity.

16. Non-human animal transformed by using the expression vector active in animals according to claim 10.

17. Non-human animal mutated at the nucleotide sequence according to any one of claims 1-6 having a reduced or inhibited silencing activity.

18. Protein characterized in having a silencing activity and comprising a *recQ* helicase domain wherein the domain is at least 30% homologous to the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

19. Protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 18 wherein the domain is at least 40% homologous to the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

20. Protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 19 wherein the domain is at least 60% homologous to the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

21. Protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 20 wherein the domain is the amino acid sequence from aa. 897 to aa. 1330 of SEQ ID No.1.

22. Protein characterized in having a silencing activity and comprising a *recQ* helicase domain according to claim 21 comprising the amino acid sequence of SEQ ID. No.1 or functional portions thereof.

23. Use of the nucleotide sequence according to any one of claims 1-6 to modulate the gene silencing in plants, animals and fungi.

24. Use of the nucleotide sequence according to any one of claims 1-6 to potentiate the antiviral-response in a plant.

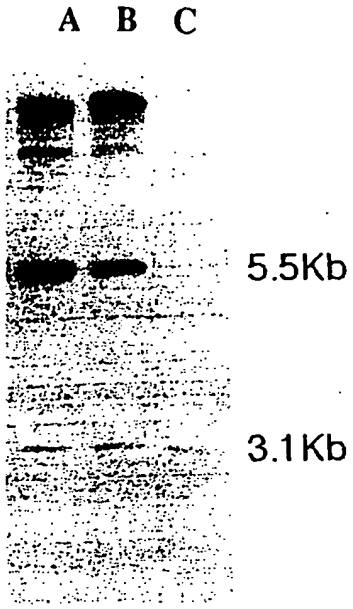
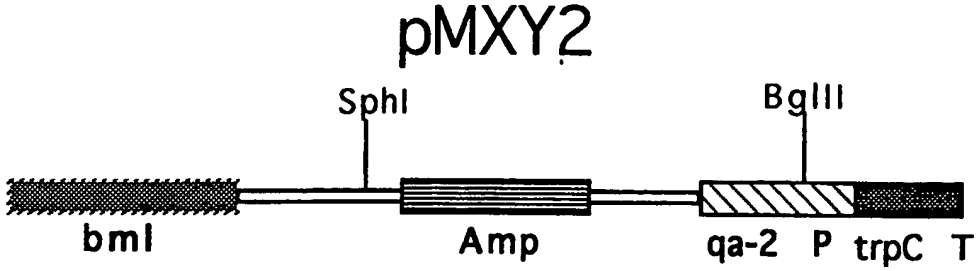


FIG. 1

FIG. 2



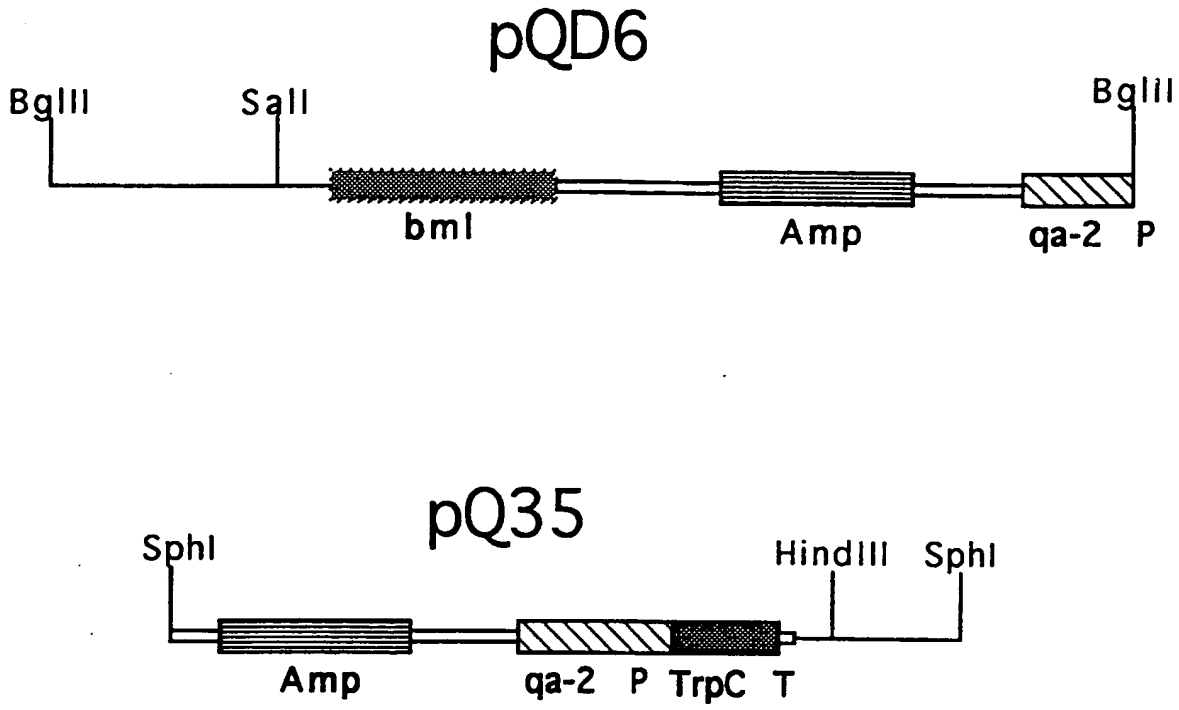

pQD6
BglII
SalI
BglII
bml
Amp
qa-2
P
pQ35
SphI
HindIII
SphI
Amp
qa-2
P
TrpC
T

FIG. 3

```
          CCA CCA CCA CCA CCA CCA CCA CAA CAA CCA ACT CAA CAA CGA ACT CAA CAA CCA
                     9          18          27          36          45          54

ACC CCA AAC CCA ACC TCG ACC TCA ACC TCA ACC TCA ACC CTT GCG ACC TCG AGA TAC ACA
          63          72          81          90          99         108

AAC ACA TCC TCG ACA AAT GAC GCC CGA CCC GCT ACA CGC CAA CAG ATT GCC CCC GAG GTA
         123         132         141         150         159         168

                                                                   M   A
GGA GCA TCG ACG CAT CAG GAT TCA GTT GGA CTT GGA GAA GGA GGA GGA GGA GGA ATG GCG
         183         192         201         210         219         228

K   L   S   V   K   N   N   L   P   R   P   H   L   V   S   L   S   S   S   T
AAG CTC TCA GTC AAG AAC AAC CTG CCA CGG CCG CAC TTG GTC TCC TTG TCG TCG TCA ACG
         243         252         261         270         279         288

T   G   S   G   S   G   S   A   S   R   S   A   S   A   K   H   G   S   A   G
ACA GGC TCT GGG TCT GGT TCT GCG TCT AGG TCA GCT TCT GCT AAG CAC GGA AGT GCC GGT
         303         312         321         330         339         348

S   S   T   F   D   H   E   Q   H   Q   Q   H   Q   Q   Q   Q   Q   Q   K   R
TCC AGT ACC TTT GAT CAT GAA CAA CAT CAA CAA CAT CAA CAA CAA CAA CAA CAA AAG CGC
         363         372         381         390         399         408

Q   R   S   Q   S   E   A   R   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q
CAG CGG TCG CAA TCA GAA GCA CGA CAA CAG CAG CAG CAA CAG CAA CAG CAA CAG CAA CAG
         423         432         441         450         459         468

Q   Q   Q   Q   Q   Q   A   Q   H   H   A   H   S   T   Y   A   Q   R   P   Q
CAA CAA CAA CAA CAA CAA GCA CAG CAC CAT GCA CAT TCT ACA TAT GCA CAA AGA CCC CAA
         483         492         501         510         519         528

P   T   P   Q   Q   R   P   P   Q   N   L   L   T   P   A   S   T   T   G   A
CCC ACC CCC CAA CAA CGA CCA CCC CAA AAC CTA CTG ACA CCT GCT TCA ACC ACT GGT GCC
         543         552         561         570         579         588

S   V   G   P   L   Q   R   A   Y   S   V   S   L   A   A   R   Q   S   P   S
AGC GTC GGC CCG CTC CAA CGC GCA TAC TCG GTT TCA TTA GCT GCG AGA CAG TCC CCC TCG
         603         612         621         630         639         648

T   N   L   V   R   P   K   T   D   S   P   A   P   H   T   L   H   L   K   N
ACA AAC TTG GTC CGT CCA AAG ACC GAC TCG CCA GCT CCC CAC ACT TTA CAC CTC AAG AAC
         663         672         681         690         699         708

K   K   N   L   R   H   P   A   P   T   P   D   S   P   I   V   D   D   D   I
AAG AAG AAC CTC CGT CAC CCC GCC CCC ACG CCC GAC AGT CCG ATC GTA GAC GAC GAT ATT
         723         732         741         750         759         768

F   S   D   A   V   D   L   T   E   E   L   D   H   D   H   D   L   N   G   K
TTC TCC GAC GCC GTC GAT CTT ACC GAA GAA CTC GAT CAT GAC CAT GAT CTC AAC GGC AAA
         783         792         801         810         819         828

D   K   D   N   T   D   N   D   N   T   V   A   S   S   S   L   I   G   F   G
GAC AAA GAC AAC ACC GAC AAC GAC AAC ACA GTC GCT TCC AGT TCG CTA ATA GGG TTC GGC
         843         852         861         870         879         888

D   D   K   L   L   W   R   E   D   F   A   E   R   A   E   P   E   H   E   R
GAT GAC AAG TTA CTG TGG CGA GAG GAC TTT GCT GAG CGT GCA GAG CCC GAA CAT GAA AGA
         903         912         921         930         939         948

G   G   S   R   P   R   Q   V   K   K   R   K   I   S   N   D   Y   I   M   K
GGT GGG AGC AGG CCT CGC CAG GTC AAG AAA CGG AAG ATA TCG AAT GAC TAC ATT ATG AAG
         963         972         981         990         999        1008

D   E   D   V   S   L   F   D   D   D   G   E   E   D   E   F   M   D   I   N
GAT GAG GAT GTC TCG CTT TTT GAT GAT GAT GGC GAG GAG GAC GAG TTT ATG GAT ATC AAT
        1023        1032        1041        1050        1059        1068
```

FIG. 4/1

```
E   L   V   Q   G   D   R   E   S   T   P   K   P   K   A   T   S   R   S   V
GAG CTA GTT CAG GGG GAT CGG GAA AGT ACT CCG AAG CCA AAG GCT ACA TCG AGG TCT GTC
       1083        1092        1101        1110        1119        1128

S   T   R   L   P   P   T   V   S   L   Q   R   G   R   S   P   K   R   K   E
TCG ACG AGG CTG CCG CCT ACA GTA TCG CTG CAA CGG GGT CGG TCT CCT AAG AGG AAG GAG
       1143        1152        1161        1170        1179        1188

A   S   V   E   K   R   T   T   E   N   Q   Q   Q   A   D   R   E   D   E   P
GCT TCA GTT GAA AAG CGC ACA ACG GAA AAC CAG CAA CAG GCT GAC AGA GAA GAC GAA CCG
       1203        1212        1221        1230        1239        1248

S   F   M   S   S   P   D   V   D   N   S   R   K   R   K   S   S   G   S   P
TCG TTT ATG TCA AGT CCA GAT GTC GAC AAC TCC CGC AAG CGA AAG TCT TCT GGA TCG CCC
       1263        1272        1281        1290        1299        1308

T   G   L   T   T   P   R   P   Q   Q   K   Q   T   E   E   V   P   G   T   T
ACA GGT TTA ACG ACG CCA AGA CCC CAG CAG AAG CAA ACG GAA GAG GTC CCA GGT ACG ACC
       1323        1332        1341        1350        1359        1368

T   A   K   K   P   R   R   S   E   V   M   D   S   E   D   E   A   F   T   P
ACC GCC AAG AAG CCA CGG CGC AGT GAA GTG ATG GAC TCG GAG GAC GAG GCA TTC ACT CCT
       1383        1392        1401        1410        1419        1428

L   S   A   G   S   L   P   G   S   A   E   F   F   R   S   G   G   T   T   T
CTT TCT GCT GGG TCG CTG CCT GGG AGT GCG GAG TTC TTC AGA AGC GGT GGG ACC ACC ACA
       1443        1452        1461        1470        1479        1488

R   E   L   G   L   D   E   D   T   V   M   D   T   P   S   R   P   P   V   E
CGG GAA TTG GGT TTG GAC GAA GAC ACG GTT ATG GAC ACG CCT AGT AGG CCA CCG GTC GAG
       1503        1512        1521        1530        1539        1548

S   T   L   P   T   L   E   S   V   E   S   R   P   P   P   L   P   P   M   D
TCC ACT TTG CCA ACT CTC GAG TCT GTG GAA AGT CGA CCA CCC CCC CTG CCG CCC ATG GAT
       1563        1572        1581        1590        1599        1608

L   P   S   Q   R   K   P   L   E   P   L   N   T   P   R   N   Q   L   L   E
CTA CCA TCA CAG CGA AAA CCG CTA GAG CCG TTG AAC ACT CCG CGC AAC CAG CTG CTT GAG
       1623        1632        1641        1650        1659        1668

S   V   E   R   P   T   Q   Q   P   S   V   G   P   S   F   A   Q   S   S   T
TCG GTC GAA AGG CCA ACA CAG CAG CCG TCG GTG GGG CCG AGT TTT GCA CAG AGT AGC ACA
       1683        1692        1701        1710        1719        1728

L   A   E   S   S   L   P   P   S   M   P   P   P   S   E   D   P   L   N   T
CTC GCC GAA AGC TCC CTG CCG CCG TCA ATG CCG CCG CCA AGT GAA GAC CCC CTC AAC ACC
       1743        1752        1761        1770        1779        1788

R   E   N   S   N   L   E   E   F   D   Y   K   L   Y   K   P   L   L   D   L
AGG GAG AAC AGC AAC CTT GAG GAG TTC GAC TAC AAG CTT TAC AAA CCC CTG CTA GAT CTT
       1803        1812        1821        1830        1839        1848

F   V   N   A   P   A   I   L   E   R   E   L   S   A   V   N   D   E   L   Q
TTC GTC AAC GCA CCC GCA ATC TTG GAA AGA GAA CTG AGC GCC GTT AAT GAC GAG CTT CAG
       1863        1872        1881        1890        1899        1908

E   N   M   I   K   L   R   D   C   L   R   L   P   R   E   E   R   D   R   A
GAG AAC ATG ATC AAG CTG CGG GAC TGT CTG CGC CTG CCC AGG GAA GAA AGA GAC AGG GCA
       1923        1932        1941        1950        1959        1968

R   E   E   V   K   K   E   K   E   M   L   K   R   R   D   I   A   L   R   A
CGC GAA GAG GTG AAG AAG GAA AAG GAA ATG CTC AAG CGA CGG GAC ATT GCG CTC AGA GCC
       1983        1992        2001        2010        2019        2028

L   Q   D   E   H   K   L   Y   V   K   K   R   K   E   H   N   L   I   N   E
CTC CAG GAC GAA CAC AAG TTG TAC GTC AAG AAA CGC AAA GAG CAT AAT TTG ATC AAC GAG
       2043        2052        2061        2070        2079        2088

E   I   V   R   A   Y   A   E   E   D   D   E   Y   E   D   Q   L   M   A   Q
GAA ATC GTT CGC GCT TAT GCT GAA GAA GAC GAT GAG TAC GAG GAT CAG TTA ATG GCG CAG
       2103        2112        2121        2130        2139        2148
```

FIG. 4/2

```
L   D   K   L   D   D   E   V   E   A   I   V   K   S   L   T   R   L   I   V
CTG GAC AAG TTG GAT GAT GAG GTT GAG GCT ATC GTA AAG AGT CTG ACA AGG CTT ATT GTG
        2163        2172        2181        2190        2199        2208

A   A   G   I   T   E   K   S   F   D   L   K   K   E   E   E   E   E   E   E
GCG GCG GGG ATC ACG GAG AAG AGC TTT GAC CTA AAG AAG GAG GAG GAA GAG GAG GAG GAG
        2223        2232        2241        2250        2259        2268

K   P   I   I   I   A   T   P   T   P   S   T   R   T   E   A   P   V   L   P
AAG CCG ATC ATC ATA GCG ACT CCG ACA CCT TCG ACG AGG ACC GAG GCC CCG GTT CTG CCG
        2283        2292        2301        2310        2319        2328

T   T   E   Y   H   N   S   Q   Q   V   I   L   Q   T   Q   H   P   A   A   Q
ACG ACC GAG TAT CAT AAT TCC CAG CAG GTC ATA TTG CAG ACT CAA CAT CCT GCT GCG CAG
        2343        2352        2361        2370        2379        2388

Q   V   S   H   R   V   P   P   P   P   T   P   S   F   Q   T   A   R   Q   T
CAG GTT TCT CAC CGG GTG CCA CCA CCT CCG ACA CCG AGT TTT CAA ACG GCG CGC CAG ACT
        2403        2412        2421        2430        2439        2448

P   V   S   Y   Q   S   R   P   T   N   N   S   F   P   D   I   S   A   E   E
CCG GTG TCA TAT CAG AGC AGA CCG ACC AAC AAC TCC TTT CCT GAT ATC TCG GCG GAA GAA
        2463        2472        2481        2490        2499        2508

A   M   M   F   D   K   E   D   P   F   M   E   Q   Q   H   A   P   A   S   A
GCC ATG ATG TTC GAT AAA GAA GAC CCC TTC ATG GAA CAA CAG CAC GCC CCG GCC TCT GCT
        2523        2532        2541        2550        2559        2568

P   F   Q   A   T   L   P   Q   R   N   S   P   F   K   T   A   P   F   K   P
CCC TTC CAG GCC ACC CTT CCC CAG CGC AAC AGC CCT TTC AAA ACC GCC CCG TTC AAG CCA
        2583        2592        2601        2610        2619        2628

V   H   G   H   D   Y   F   D   D   E   D   D   D   A   D   L   L   A   A   V
GTC CAC GGC CAC GAT TAC TTT GAC GAT GAA GAC GAC GAT GCC GAC CTC CTG GCA GCA GTA
        2643        2652        2661        2670        2679        2688

D   S   A   E   T   Y   T   S   T   A   A   T   T   T   T   N   N   N   N   H
GAC AGC GCC GAG ACG TAT ACT TCT ACG GCC GCC ACC ACC ACC ACC AAC AAC AAC AAT CAC
        2703        2712        2721        2730        2739        2748

L   R   S   Q   S   V   M   S   T   S   T   A   T   T   I   K   P   R   K   R
TTA CGA TCA CAA TCG GTG ATG TCA ACA TCC ACG GCG ACC ACG ATC AAA CCG AGG AAA CGC
        2763        2772        2781        2790        2799        2808

N   E   N   A   N   A   K   K   P   K   S   V   H   A   K   L   S   M   P   P
AAC GAA AAT GCC AAT GCC AAG AAG CCC AAG TCC GTA CAT GCA AAG TTA TCG ATG CCG CCC
        2823        2832        2841        2850        2859        2868

E   K   M   K   Y   A   W   S   N   D   V   R   K   A   L   K   D   R   F   R
GAA AAG ATG AAG TAT GCG TGG TCG AAT GAT GTG AGG AAG GCT CTC AAG GAT AGG TTT CGG
        2883        2892        2901        2910        2919        2928

M   S   G   F   R   Q   N   Q   L   E   A   I   N   A   T   L   G   G   K
ATG TCG GGG TTC AGA CAG AAT CAG TTG GAG GCT ATT AAT GCT ACT TTG GGT GGT AAG GTG
        2943        2952        2961        2970        2979        2988

AGT TCT CTG TCC TTT ACC TAT CTG GGA GAG ACC AAG AAG GAG AGA GAG AGA GAG AGG AGG
        3003        3012        3021        3030        3039        3048

                                                D   A   F   V   L   M   P   T   G
GGA AGA CGA AAA TGG ACT TTG CTG ACT CTA GAAAG GAT GCC TTT GTG TTG ATG CCG ACT GGT
        3065        3074        3083        3092        3101        3110

G   G   K   S   L   C   Y   Q   L   P   A   V   V   R   S   G   K   T   R   G
GGT GGA AAG TCT CTG TGC TAT CAG TTG CCG GCT GTA GTC AGG AGC GGC AAG ACG CGT GGT
        3125        3134        3143        3152        3161        3170

I   T   V   V   I   S   P   L   L   S   L   M   L   D   Q   V   N   H   L   A
ATC ACA GTC GTC ATC TCC CCT CTG CTA AGT CTG ATG CTG GAT CAA GTC AAC CAT TTG GCA
        3185        3194        3203        3212        3221        3230
```

FIG. 4/3

```
N  L  M  I  Q  A  Y  A  F  N  G  D  M  N  S  E  M  R  R  M
AAC CTG ATG ATC CAA GCT TAC GCT TTC AAC GGA GAC ATG AAC TCA GAA ATG CGC CGA ATG
      3245       3254       3263       3272       3281       3290

V  F  Q  K  L  D  A  E  H  P  E  H  E  L  Q  L  L  Y  V  T
GTG TTT CAG AAG CTT GAT GCT GAG CAT CCT GAG CAT GAG CTC CAA CTG CTC TAT GTC ACC
      3305       3314       3323       3332       3341       3350

P  E  M  V  S  K  N  Q  T  F  V  N  K  M  M  D  L  Y  R  R
CCG GAG ATG GTG AGC AAG AAC CAG ACA TTC GTC AAC AAG ATG ATG GAC CTC TAC CGG AGG
      3365       3374       3383       3392       3401       3410

K  K  L  A  R  I  V  I  D  E  A  H  C  V  S  Q  W  G  H  D
AAA AAG CTG GCT AGA ATT GTT ATC GAC GAG GCT CAC TGC GTC AGT CAA TGG GGC CAT GAC
      3425       3434       3443       3452       3461       3470

F  R  P  D  Y  K  A  I  G  E  F  R  K  R  F  P  G  V  P  V
TTC CGA CCC GAT TAC AAA GCT ATA GGA GAG TTT CGT AAG AGG TTT CCC GGA GTT CCG GTC
      3485       3494       3503       3512       3521       3530

M  A  L  T  A  T  A  T  Q  N  V  I  L  D  V  K  H  N  L  A
ATG GCT TTG ACA GCG ACG GCA ACA CAG AAC GTC ATC TTG GAT GTC AAG CAT AAC CTG GCA
      3545       3554       3563       3572       3581       3590

M  E  D  C  Q  T  F  S  Q  S  F  N  R  P  N  L  Y  Y  E  V
ATG GAG GAC TGC CAG ACT TTC TCC CAG AGC TTT AAT CGG CCG AAC CTC TAC TAT GAG GTC
      3605       3614       3623       3632       3641       3650

R  M  K  E  Q  N  L  I  A  R  I  A  E  L  I  K  E  K  Y  D
AGG ATG AAG GAG CAG AAT CTG ATT GCC CGC ATC GCA GAG TTG ATC AAG GAG AAG TAT GAC
      3665       3674       3683       3692       3701       3710

G  Q  T  G  I  I  Y  T  L  S  R  K  S  A  E  N  I  A  K  N
GGC CAG ACG GGT ATC ATC TAC ACA TTA TCA AGA AAG AGT GCC GAG AAC ATC GCC AAA AAT
      3725       3734       3743       3752       3761       3770

L  Q  E  K  H  R  I  K  A  K  H  Y  H  A  S  I  T  T  D  E
CTC CAG GAA AAA CAC CGC ATC AAA GCA AAG CAC TAC CAT GCC TCC ATC ACC ACC GAC GAA
      3785       3794       3803       3812       3821       3830

K  I  S  V  Q  H  E  W  Q  T  G  R  V  K  V  V  V  A  T  I
AAG ATC AGC GTT CAA CAT GAA TGG CAA ACC GGC CGA GTC AAA GTC GTG GTA GCC ACC ATT
      3845       3854       3863       3872       3881       3890

A  F  G  M  G  I  D  K  P  D  V  R  F  V  I  H  Q  H  I  P
GCC TTC GGC ATG GGC ATC GAC AAG CCT GAC GTC CGC TTT GTT ATC CAC CAG CAC ATC CCC
      3905       3914       3923       3932       3941       3950

K  S  L  E  G  Y  Y  Q  E  T  G  R  A  G  R  D  G  K  P  S
AAG TCG CTC GAA GGT TAC TAT CAA GAA ACC GGC CGC GCC GGA CGT GAC GGC AAG CCA TCG
      3965       3974       3983       3992       4001       4010

D  C  Y  L  Y  F  A  Y  G  D  I  Q  S  L  R  R  M  I  A  D
GAC TGC TAC TTG TAC TTT GCC TAT GGC GAC ATT CAA TCC CTA CGT CGT ATG ATC GCC GAC
      4025       4034       4043       4052       4061       4070

G  E  G  D  Y  A  Q  K  E  R  Q  L  Q  M  L  N  R  V  V  S
GGC GAA GGT GAC TAC GCG CAA AAG GAG CGT CAG CTA CAA ATG CTC AAC CGT GTG GTC AGC
      4085       4094       4103       4112       4121       4130

Y  C  E  S  Q  H  T  C  R  R  E  E  V  L  R  Y  F  G  E  E
TAT TGC GAG TCG CAG CAC ACG TGC CGG CGC GAA GAA GTG CTC CGC TAC TTT GGC GAG GAG
      4145       4154       4163       4172       4181       4190

F  D  Y  R  K  C  R  D  G  C  D  N  C  R  N  G  R  I  S  K
TTT GAC TAC CGG AAG TGT AGA GAC GGA TGC GAT AAC TGC CGG AAC GGA CGC ATC TCG AAG
      4205       4214       4223       4232       4241       4250

S  T  E  M  R  D  F  T  E  I  A  F  A  A  I  E  V  V  K  S
TCG ACG GAG ATG AGG GAT TTT ACG GAA ATC GCC TTC GCC GCG ATC GAG GTG GTG AAG AGC
      4265       4274       4283       4292       4301       4310
```

FIG 4/4

```
Q   Q   P   I   T   L   G   K   L   C   D   I   L   M   G   K   R   K   N   E
CAG CAG CCC ATC ACG CTG GGC AAG CTG TGC GAC ATC CTG ATG GGC AAG AGA AAG AAC GAG
        4325        4334        4343        4352        4361        4370

H   G   G   V   C   H   F   G   I   A   K   G   S   T   Q   R   E   L   Q   R
CAC GGT GGC GTG TGT CAC TTT GGT ATC GCC AAG GGG AGC ACG CAG AGG GAG CTG CAG AGG
        4385        4394        4403        4412        4421        4430

I   V   L   Q   L   N   F   H   K   A   L   G   E   D   N   I   M   N   G   A
ATC GTG CTG CAG CTG AAT TTC CAC AAG GCG CTG GGC GAG GAC AAT ATC ATG AAT GGG GCG
        4445        4454        4463        4472        4481        4490

G   M   P   I   T   Y   Y   I
GGG ATG CCT ATT ACC TAC TAT ATT GTG AGT GCT GTC CCG GTT GGT CTT GCA TAT CTG GCT
        4505        4514        4523        4532        4541        4550

                                                        /INSERZIONE
                                                T   G   P   E   A   G   A   Y   L
TTG TTG CTT TGC TAA CAC AGC AGC TCG TAC AG ACC GGC CCT GAA GCT GGT GCT TAC CTC
        4564        4573        4582        4591        4600        4609

Y   N   G   K   R   L   M   L   P   V   P   S   N   K   S   V   E   P   P   S
TAC AAT GGC AAG CGG TTG ATG CTG CCA GTT CCC TCA AAC AAG TCC GTC GAA CCC CCG TCT
        4624        4633        4642        4651        4660        4669

R   S   K   Q   R   S   R   R   V   D   E   D   M   D   E   Q   E   L   S   T
CGG TCT AAG CAG CGG AGC CGT CGA GTC GAT GAG GAT ATG GAT GAG CAA GAA CTT TCC ACC
        4684        4693        4702        4711        4720        4729

L   Q   R   P   P   T   S   T   N   V   S   S   P   V   R   A   T   K   K   R
CTG CAA CGA CCG CCA ACA TCA ACA AAT GTC TCT TCA CCC GTT CGA GCC ACC AAG AAA CGA
        4744        4753        4762        4771        4780        4789

S   S   K   K   A   L   P   T   L   I   A   D   Y   E   E   P   S   S   D   G
AGT TCC AAA AAG GCT TTA CCG ACC CTC ATC GCC GAC TAC GAA GAG CCC AGC TCC GAC GGT
        4804        4813        4822        4831        4840        4849

P   H   G   P   L   H   A   N   G   Y   E   R   D   N   F   V   V   S   D   N
CCT CAC GGT CCT CTC CAC GCC AAC GGC TAT GAG CGC GAT AAC TTT GTC GTA TCC GAT AAT
        4864        4873        4882        4891        4900        4909

V   E   P   E   E   E   E   D   A   F   E   P   V   R   P   S   R   R   G   P
GTT GAA CCC GAA GAG GAA GAA GAT GCC TTC GAA CCT GTC CGC CCC TCG CGG CGC GGC CCA
        4924        4933        4942        4951        4960        4969

S   S   R   A   T   R   P   Q   H   R   Q   T   T   L   Y   D   T   L   S   H
TCT TCT CGC GCT ACC CGC CCT CAA CAC CGC CAG ACC ACC CTT TAT GAC ACC CTC TCC CAC
        4984        4993        5002        5011        5020        5029

T   Q   Q   S   Q   T   V   S   Q   H   L   A   T   L   G   P   P   I   D   A
ACC CAA CAA TCC CAA ACC GTC TCC CAA CAC CTC GCC ACT TTG GGT CCG CCC ATC GAC GCC
        5044        5053        5062        5071        5080        5089

R   T   M   H   N   P   R   Y   A   Q   L   D   E   V   H   Q   D   I   V   D
CGC ACC ATG CAT AAC CCC CGC TAC GCC CAG CTT GAC GAG GTC CAC CAG GAT ATT GTC GAT
        5104        5113        5122        5131        5140        5149

A   F   V   E   E   V   K   V   F   E   E   D   F   R   N   R   N   H   M   R
GCC TTT GTT GAA GAA GTC AAG GTC TTC GAG GAG GAC TTT CGC AAC AGG AAC CAC ATG CGC
        5164        5173        5182        5191        5200        5209

K   P   I   F   T   E   T   Q   Y   R   E   M   A   I   R   W   T   R   S   L
AAA CCC ATC TTT ACC GAG ACG CAG TAC CGT GAG ATG GCA ATC CGG TGG ACG CGG TCG TTA
        5224        5233        5242        5251        5260        5269

D   A   M   R   A   I   P   D   I   N   Q   D   K   V   D   R   Y   G   A   K
GAC GCG ATG CGC GCG ATC CCG GAT ATC AAC CAG GAT AAA GTA GAT CGG TAT GGT GCC AAA
        5284        5293        5302        5311        5320        5329

F   I   P   L   V   E   R   F   W   G   N   Y   Q   E   M   M   G   G   G   Y
TTC ATC CCA CTT GTG GAG CGG TTC TGG GGG AAT TAT CAG GAG ATG ATG GGG GGA GGG TAT
        5344        5353        5362        5371        5380        5389
```

FIG 4/5

```
D   N   P   A   V   A   G   D   E   D   D   D   E   G   P   R   R   T   G   N
GAT AAT CCT GCT GTG GCT GGC GAT GAG GAT GAT GAT GAG GGC CCC AGG AGG ACA GGA AAT
        5404        5413        5422        5431        5440        5449

G   K   G   G   N   K   K   G   G   G   G   G   G   G   N   E   V   V   D   L
GGA AAA GGG GGG AAT AAG AAG GGA GGA GGA GGA GGA GGA GGA AAT GAA GTA GTG GAT TTG
        5464        5473        5482        5491        5500        5509

I   S   S   D   E   D   E   P   P   A   R   A   P   S   R   N   A   G   R   G
ATT AGT AGT GAT GAG GAT GAA CCT CCG GCT CGT GCA CCA TCG CGG AAT GCG GGG CGA GGA
        5524        5533        5542        5551        5560        5569

K   A   Q   S   T   R   G   G   Q   I   Q   D   K   G   R   A   V   N   R   R
AAG GCA CAG TCG ACA CGT GGG GGA CAA ATC CAA GAT AAA GGC CGA GCA GTC AAC CGC CGC
        5584        5593        5602        5611        5620        5629

G   E   P   I   A   E   E   D   E   E   D   Y   G   L   S   D   P   D   I   D
GGA GAA CCC ATC GCC GAA GAA GAC GAA GAA GAC TAC GGG CTA AGC GAC CCC GAT ATC GAC
        5644        5653        5662        5671        5680        5689

A   I   D   P   D   A   I   T   A   S   D   N   S   D   E   E   D   D   D   D
GCC ATC GAT CCA GAC GCC ATC ACC GCC TCC GAC AAC TCC GAC GAA GAA GAT GAT GAT GAT
        5704        5713        5722        5731        5740        5749

D   D   E   D   L   E   S   S   R   Y   F   S   G   S   T   G   P   P   V   S
GAT GAC GAA GAC CTC GAA TCC TCC CGC TAC TTC TCC GGC TCA ACA GGC CCG CCC GTC TCC
        5764        5773        5782        5791        5800        5809

K   A   V   Q   D   A   R   L   R   E   Q   L   S   M   Y   A   S   G   G   S
AAA GCC GTG CAG GAT GCT CGA CTC CGT GAA CAA CTT TCC ATG TAC GCC TCC GGC GGC AGC
        5824        5833        5842        5851        5860        5869

S   S   K   G   S   Y   G   S   G   R   A   S   G   G   S   S   S   R   A   S
TCT TCG AAA GGT AGC TAC GGC TCA GGG CGC GCA TCA GGA GGA TCT TCG TCG AGA GCG TCG
        5884        5893        5902        5911        5920        5929

G   S   G   W   R   G   G   G   A   G   G   K   K   Y   Y   R   K   K   R   A
GGA TCA GGA TGG AGA GGT GGA GGA GCA GGT GGG AAG AAA TAC TAC AGG AAG AAG AGG GCT
        5944        5953        5962        5971        5980        5989

G   S   S   A   A   G   G   G   A   G   G   G   G   V   T   K   R   K   A
GGT TCT TCG GCT GCT GGT GGT GGT GGT GCA GGA GGA GGG GGA GTT ACA AAA CGG AAG GCG
        6004        6013        6022        6031        6040        6049

S   G   S   G   A   K   T   A   R   K   R   G   A   S   T   A   P   K   T   T
AGT GGG AGT GGC GCG AAG ACG GCG AGG AAG AGG GGT GCA TCT ACT GCG CCG AAG ACA ACG
        6064        6073        6082        6091        6100        6109

T   R   G   G   G   S   G   A   G   S   R   G   G   G   A   G   G   A   G   G
ACG AGA GGG GGA GGA TCT GGA GCT GGG TCT AGA GGA GGC GGT GCT GGT GGT GCT GGT GGT
        6124        6133        6142        6151        6160        6169

A   G   A   G   A   G   A   G   G   G   K   R   G   G   G   G   G   G   G   M
GCT GGT GCT GGT GCT GGT GCT GGA GGA GGG AAA AGG GGT GGT GGA GGT GGA GGA GGA ATG
        6184        6193        6202        6211        6220        6229

G   G   I   S   V   M   P   H   .
GGA GGG ATA AGT GTT ATG CCT CAT TAG CTA TTT TAT AGC ATA TCG CAT TTA TAC AGT GTC
        6244        6253        6262        6271        6280        6289

TTA TGG AAG GGA GGA GGA GAA GAA GAA GGA TAA GCT GGC ATA AGC TTG AAC CGG CCA GGC
        6304        6313        6322        6331        6340        6349

CAA AAT GGC CAG AGA GCT CAC CGG GCA ATC GAG CTT GAA ATG AGC TTG ACA TAT TAG GTA
        6364        6373        6382        6391        6400        6409

TTC CCG AGA ATA TAG CGG GAT TAC AAG GCA CTT ACT TTA CCA AGT CGA AAG GGA CGA GCC
        6424        6433        6442        6451        6460        6469

AAA TCT ATG GTA CTC GCC AGT TGC GCA ACG TTG AGT TTT ATC ATT CGT GGA GTT TTC ATC
        6484        6493        6502        6511        6520        6529
```

FIG. 4/6

```
GTG GAG TTT TTA TTA TCA ACT ATT CGT TGT ATA GTT TTC GTT GTA GAT GTT AGT TCC GGA
      6544        6553        6562        6571        6580        6589

CGA TCA AAA GGG GAA GTG TGG AAC AGA GAA GTC GAA AGG ACA AGC CAA AAT GAC ATG GCA
      6604        6613        6622        6631        6640        6649

GTG TCC AGT CAG ATA CCC TCC AGA CAA AAC CAG ACA CCA ATA ACA AAC CCT TCA ACC ATA
      6664        6673        6682        6691        6700        6709

ACA CCA GCA AAG CCA ATC CTT AGG TAC CTA CCT AGG GTA GGG TAG GTC CAG GAA TGT CTT
      6724        6733        6742        6751        6760        6769

CCC CAA AGG TAC CTC TAC TTA TTC ATG TTA CGC TCC ATC AGT CCC ATC GCT TAG CAT CGC
      6784        6793        6802        6811        6820        6829

TGC CCG GTT ACC TAT CTC TAC CTC TAC CTC TAC CTC TAC CTC TAC CTC TAC CTC TAC CTC
      6844        6853        6862        6871        6880        6889

TAT CTC TAC CTC TAC CTC TAC
      6904        6913
```

FIG. 4/7

SEQ ID No.1

```
          M   A   K   L   S   V   K   N   N   L   P   R   P   H   L   V   S   L
          ATG GCG AAG CTC TCA GTC AAG AAC AAC CTG CCA CGG CCG CAC TTG GTC TCC TTG
                    9          18          27          36          45          54

S   S   S   T   T   G   S   G   S   G   S   A   S   R   S   A   S   A   K   H
TCG TCG TCA ACG ACA GGC TCT GGG TCT GGT TCT GCG TCT AGG TCA GCT TCT GCT AAG CAC
      63          72          81          90          99          108

G   S   A   G   S   S   T   F   D   H   E   Q   H   Q   Q   H   Q   Q   Q   Q
GGA AGT GCC GGT TCC AGT ACC TTT GAT CAT GAA CAA CAT CAA CAA CAT CAA CAA CAA CAA
      123         132         141         150         159         168

Q   Q   K   R   Q   R   S   Q   S   E   A   R   Q   Q   Q   Q   Q   Q   Q   Q
CAA CAA AAG CGC CAG CGG TCG CAA TCA GAA GCA CGA CAA CAG CAG CAG CAA CAG CAA CAG
      183         192         201         210         219         228

Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   A   Q   H   H   A   H   S   T   Y   A
CAA CAG CAA CAG CAA CAA CAA CAA CAA CAA GCA CAG CAC CAT GCA CAT TCT ACA TAT GCA
      243         252         261         270         279         288

Q   R   P   Q   P   T   P   Q   Q   R   P   P   Q   N   L   L   T   P   A   S
CAA AGA CCC CAA CCC ACC CCC CAA CAA CGA CCA CCC CAA AAC CTA CTG ACA CCT GCT TCA
      303         312         321         330         339         348

T   T   G   A   S   V   G   P   L   Q   R   A   Y   S   V   S   L   A   A   R
ACC ACT GGT GCC AGC GTC GGC CCG CTC CAA CGC GCA TAC TCG GTT TCA TTA GCT GCG AGA
      363         372         381         390         399         408

Q   S   P   S   T   N   L   V   R   P   K   T   D   S   P   A   P   H   T   L
CAG TCC CCC TCG ACA AAC TTG GTC CGT CCA AAG ACC GAC TCG CCA GCT CCC CAC ACT TTA
      423         432         441         450         459         468

H   L   K   N   K   K   N   L   R   H   P   A   P   T   P   D   S   P   I   V
CAC CTC AAG AAC AAG AAG AAC CTC CGT CAC CCC GCC CCC ACG CCC GAC AGT CCG ATC GTA
      483         492         501         510         519         528

D   D   D   I   F   S   D   A   V   D   L   T   E   E   L   D   H   D   H   D
GAC GAC GAT ATT TTC TCC GAC GCC GTC GAT CTT ACC GAA GAA CTC GAT CAT GAC CAT GAT
      543         552         561         570         579         588

L   N   G   K   D   K   D   N   T   D   N   D   N   T   V   A   S   S   S   L
CTC AAC GGC AAA GAC AAA GAC AAC ACC GAC AAC GAC AAC ACA GTC GCT TCC AGT TCG CTA
      603         612         621         630         639         648

I   G   F   G   D   D   K   L   L   W   R   E   D   F   A   E   R   A   E   P
ATA GGG TTC GGC GAT GAC AAG TTA CTG TGG CGA GAG GAC TTT GCT GAG CGT GCA GAG CCC
      663         672         681         690         699         708

E   H   E   R   G   G   S   R   P   R   Q   V   K   K   R   K   I   S   N   D
GAA CAT GAA AGA GGT GGG AGC AGG CCT CGC CAG GTC AAG AAA CGG AAG ATA TCG AAT GAC
      723         732         741         750         759         768

Y   I   M   K   D   E   D   V   S   L   F   D   D   D   G   E   E   D   E   F
TAC ATT ATG AAG GAT GAG GAT GTC TCG CTT TTT GAT GAT GAT GGC GAG GAG GAC GAG TTT
      783         792         801         810         819         828

M   D   I   N   E   L   V   Q   G   D   R   E   S   T   P   K   P   K   A   T
ATG GAT ATC AAT GAG CTA GTT CAG GGG GAT CGG GAA AGT ACT CCG AAG CCA AAG GCT ACA
      843         852         861         870         879         888
```

FIG. 5/1

```
S   R   S   V   S   T   R   L   P   P   T   V   S   L   Q   R   G   R   S   P
TCG AGG TCT GTC TCG ACG AGG CTG CCG CCT ACA GTA TCG CTG CAA CGG GGT CGG TCT CCT
        903         912         921         930         939         948

K   R   K   E   A   S   V   E   K   R   T   T   E   N   Q   Q   Q   A   D   R
AAG AGG AAG GAG GCT TCA GTT GAA AAG CGC ACA ACG GAA AAC CAG CAA CAG GCT GAC AGA
        963         972         981         990         999        1008

E   D   E   P   S   F   M   S   S   P   D   V   D   N   S   R   K   R   K   S
GAA GAC GAA CCG TCG TTT ATG TCA AGT CCA GAT GTC GAC AAC TCC CGC AAG CGA AAG TCT
       1023        1032        1041        1050        1059        1068

S   G   S   P   T   G   L   T   T   P   R   P   Q   Q   K   Q   T   E   E   V
TCT GGA TCG CCC ACA GGT TTA ACG ACG CCA AGA CCC CAG CAG AAG CAA ACG GAA GAG GTC
       1083        1092        1101        1110        1119        1128

P   G   T   T   T   A   K   K   P   R   R   S   E   V   M   D   S   E   D   E
CCA GGT ACG ACC ACC GCC AAG AAG CCA CGG CGC AGT GAA GTG ATG GAC TCG GAG GAC GAG
       1143        1152        1161        1170        1179        1188

A   F   T   P   L   S   A   G   S   L   P   G   S   A   E   F   F   R   S   G
GCA TTC ACT CCT CTT TCT GCT GGG TCG CTG CCT GGG AGT GCG GAG TTC TTC AGA AGC GGT
       1203        1212        1221        1230        1239        1248

G   T   T   T   R   E   L   G   L   D   E   D   T   V   M   D   T   P   S   R
GGG ACC ACC ACA CGG GAA TTG GGT TTG GAC GAA GAC ACG GTT ATG GAC ACG CCT AGT AGG
       1263        1272        1281        1290        1299        1308

P   P   V   E   S   T   L   P   T   L   E   S   V   E   S   R   P   P   P   L
CCA CCG GTC GAG TCC ACT TTG CCA ACT CTC GAG TCT GTG GAA AGT CGA CCA CCC CCC CTG
       1323        1332        1341        1350        1359        1368

P   P   M   D   L   P   S   Q   R   K   P   L   E   P   L   N   T   P   R   N
CCG CCC ATG GAT CTA CCA TCA CAG CGA AAA CCG CTA GAG CCG TTG AAC ACT CCG CGC AAC
       1383        1392        1401        1410        1419        1428

Q   L   L   E   S   V   E   R   P   T   Q   Q   P   S   V   G   P   S   F   A
CAG CTG CTT GAG TCG GTC GAA AGG CCA ACA CAG CAG CCG TCG GTG GGG CCG AGT TTT GCA
       1443        1452        1461        1470        1479        1488

Q   S   S   T   L   A   E   S   S   L   P   P   S   M   P   P   P   S   E   D
CAG AGT AGC ACA CTC GCC GAA AGC TCC CTG CCG CCG TCA ATG CCG CCG CCA AGT GAA GAC
       1503        1512        1521        1530        1539        1548

P   L   N   T   R   E   N   S   N   L   E   E   F   D   Y   K   L   Y   K   P
CCC CTC AAC ACC AGG GAG AAC AGC AAC CTT GAG GAG TTC GAC TAC AAG CTT TAC AAA CCC
       1563        1572        1581        1590        1599        1608

L   L   D   L   F   V   N   A   P   A   I   L   E   R   E   L   S   A   V   N
CTG CTA GAT CTT TTC GTC AAC GCA CCC GCA ATC TTG GAA AGA GAA CTG AGC GCC GTT AAT
       1623        1632        1641        1650        1659        1668

D   E   L   Q   E   N   M   I   K   L   R   D   C   L   R   L   P   R   E   E
GAC GAG CTT CAG GAG AAC ATG ATC AAG CTG CGG GAC TGT CTG CGC CTG CCC AGG GAA GAA
       1683        1692        1701        1710        1719        1728

R   D   R   A   R   E   E   V   K   K   E   K   E   M   L   K   R   R   D   I
AGA GAC AGG GCA CGC GAA GAG GTG AAG AAG GAA AAG GAA ATG CTC AAG CGA CGG GAC ATT
       1743        1752        1761        1770        1779        1788

A   L   R   A   L   Q   D   E   H   K   L   Y   V   K   K   R   K   E   H   N
GCG CTC AGA GCC CTC CAG GAC GAA CAC AAG TTG TAC GTC AAG AAA CGC AAA GAG CAT AAT
       1803        1812        1821        1830        1839        1848
```

FIG. 5/2

```
L   I   N   E   E   I   V   R   A   Y   A   E   E   D   D   E   Y   E   D   Q
TTG ATC AAC GAG GAA ATC GTT CGC GCT TAT GCT GAA GAA GAC GAT GAG TAC GAG GAT CAG
        1863        1872        1881        1890        1899        1908

L   M   A   Q   L   D   K   L   D   D   E   V   E   A   I   V   K   S   L   T
TTA ATG GCG CAG CTG GAC AAG TTG GAT GAT GAG GTT GAG GCT ATC GTA AAG AGT CTG ACA
        1923        1932        1941        1950        1959        1968

R   L   I   V   A   A   G   I   T   E   K   S   F   D   L   K   K   E   E   E
AGG CTT ATT GTG GCG GCG GGG ATC ACG GAG AAG AGC TTT GAC CTA AAG AAG GAG GAG GAA
        1983        1992        2001        2010        2019        2028

E   E   E   E   K   P   I   I   I   A   T   P   T   P   S   T   R   T   E   A
GAG GAG GAG GAG AAG CCG ATC ATC ATA GCG ACT CCG ACA CCT TCG ACG AGG ACC GAG GCC
        2043        2052        2061        2070        2079        2088

P   V   L   P   T   T   E   Y   H   N   S   Q   Q   V   I   L   Q   T   Q   H
CCG GTT CTG CCG ACG ACC GAG TAT CAT AAT TCC CAG CAG GTC ATA TTG CAG ACT CAA CAT
        2103        2112        2121        2130        2139        2148

P   A   A   Q   Q   V   S   H   R   V   P   P   P   P   T   P   S   F   Q   T
CCT GCT GCG CAG CAG GTT TCT CAC CGG GTG CCA CCA CCT CCG ACA CCG AGT TTT CAA ACG
        2163        2172        2181        2190        2199        2208

A   R   Q   T   P   V   S   Y   Q   S   R   P   T   N   N   S   F   P   D   I
GCG CGC CAG ACT CCG GTG TCA TAT CAG AGC AGA CCG ACC AAC AAC TCC TTT CCT GAT ATC
        2223        2232        2241        2250        2259        2268

S   A   E   E   A   M   M   F   D   K   E   D   P   F   M   E   Q   Q   H   A
TCG GCG GAA GAA GCC ATG ATG TTC GAT AAA GAA GAC CCC TTC ATG GAA CAA CAG CAC GCC
        2283        2292        2301        2310        2319        2328

P   A   S   A   P   F   Q   A   T   L   P   Q   R   N   S   P   F   K   T   A
CCG GCC TCT GCT CCC TTC CAG GCC ACC CTT CCC CAG CGC AAC AGC CCT TTC AAA ACC GCC
        2343        2352        2361        2370        2379        2388

P   F   K   P   V   H   G   H   D   Y   F   D   D   E   D   D   D   A   D   L
CCG TTC AAG CCA GTC CAC GGC CAC GAT TAC TTT GAC GAT GAA GAC GAC GAT GCC GAC CTC
        2403        2412        2421        2430        2439        2448

L   A   A   V   D   S   A   E   T   Y   T   S   T   A   A   T   T   T   T   N
CTG GCA GCA GTA GAC AGC GCC GAG ACG TAT ACT TCT ACG GCC GCC ACC ACC ACC ACC AAC
        2463        2472        2481        2490        2499        2508

N   N   N   H   L   R   S   Q   S   V   M   S   T   S   T   A   T   T   I   K
AAC AAC AAT CAC TTA CGA TCA CAA TCG GTG ATG TCA ACA TCC ACG GCG ACC ACG ATC AAA
        2523        2532        2541        2550        2559        2568

P   R   K   R   N   E   N   A   N   A   K   K   P   K   S   V   H   A   K   L
CCG AGG AAA CGC AAC GAA AAT GCC AAT GCC AAG AAG CCC AAG TCC GTA CAT GCA AAG TTA
        2583        2592        2601        2610        2619        2628

S   M   P   P   E   K   M   K   Y   A   W   S   N   D   V   R   K   A   L   K
TCG ATG CCG CCC GAA AAG ATG AAG TAT GCG TGG TCG AAT GAT GTG AGG AAG GCT CTC AAG
        2643        2652        2661        2670        2679        2688

D   R   F   R   M   S   G   F   R   Q   N   Q   L   E   A   I   N   A   T   L
GAT AGG TTT CGG ATG TCG GGG TTC AGA CAG AAT CAG TTG GAG GCT ATT AAT GCT ACT TTG
        2703        2712        2721        2730        2739        2748

G   G   K   D   A   F   V   L   M   P   T   G   G   G   K   S   L   C   Y   Q
GGT GGT AAG GAT GCC TTT GTG TTG ATG CCG ACT GGT GGT GGA AAG TCT CTG TGC TAT CAG
        2763        2772        2781        2790        2799        2808
```

FIG. 5/3

```
L   P   A   V   V   R   S   G   K   T   R   G   I   T   V   V   I   S   P   L
TTG CCG GCT GTA GTC AGG AGC GGC AAG ACG CGT GGT ATC ACA GTC GTC ATC TCC CCT CTG
        2823        2832        2841        2850        2859        2868

L   S   L   M   L   D   Q   V   N   H   L   A   N   L   M   I   Q   A   Y   A
CTA AGT CTG ATG CTG GAT CAA GTC AAC CAT TTG GCA AAC CTG ATG ATC CAA GCT TAC GCT
        2883        2892        2901        2910        2919        2928

F   N   G   D   M   N   S   E   M   R   R   M   V   F   Q   K   L   D   A   E
TTC AAC GGA GAC ATG AAC TCA GAA ATG CGC CGA ATG GTG TTT CAG AAG CTT GAT GCT GAG
        2943        2952        2961        2970        2979        2988

H   P   E   H   E   L   Q   L   L   Y   V   T   P   E   M   V   S   K   N   Q
CAT CCT GAG CAT GAG CTC CAA CTG CTC TAT GTC ACC CCG GAG ATG GTG AGC AAG AAC CAG
        3003        3012        3021        3030        3039        3048

T   F   V   N   K   M   M   D   L   Y   R   R   K   K   L   A   R   I   V   I
ACA TTC GTC AAC AAG ATG ATG GAC CTC TAC CGG AGG AAA AAG CTG GCT AGA ATT GTT ATC
        3063        3072        3081        3090        3099        3108

D   E   A   H   C   V   S   Q   W   G   H   D   F   R   P   D   Y   K   A   I
GAC GAG GCT CAC TGC GTC AGT CAA TGG GGC CAT GAC TTC CGA CCC GAT TAC AAA GCT ATA
        3123        3132        3141        3150        3159        3168

G   E   F   R   K   R   F   P   G   V   P   V   M   A   L   T   A   T   A   T
GGA GAG TTT CGT AAG AGG TTT CCC GGA GTT CCG GTC ATG GCT TTG ACA GCG ACG GCA ACA
        3183        3192        3201        3210        3219        3228

Q   N   V   I   L   D   V   K   H   N   L   A   M   E   D   C   Q   T   F   S
CAG AAC GTC ATC TTG GAT GTC AAG CAT AAC CTG GCA ATG GAG GAC TGC CAG ACT TTC TCC
        3243        3252        3261        3270        3279        3288

Q   S   F   N   R   P   N   L   Y   Y   E   V   R   M   K   E   Q   N   L   I
CAG AGC TTT AAT CGG CCG AAC CTC TAC TAT GAG GTC AGG ATG AAG GAG CAG AAT CTG ATT
        3303        3312        3321        3330        3339        3348

A   R   I   A   E   L   I   K   E   K   Y   D   G   Q   T   G   I   I   Y   T
GCC CGC ATC GCA GAG TTG ATC AAG GAG AAG TAT GAC GGC CAG ACG GGT ATC ATC TAC ACA
        3363        3372        3381        3390        3399        3408

L   S   R   K   S   A   E   N   I   A   K   N   L   Q   E   K   H   R   I   K
TTA TCA AGA AAG AGT GCC GAG AAC ATC GCC AAA AAT CTC CAG GAA AAA CAC CGC ATC AAA
        3423        3432        3441        3450        3459        3468

A   K   H   Y   H   A   S   I   T   T   D   E   K   I   S   V   Q   H   E   W
GCA AAG CAC TAC CAT GCC TCC ATC ACC ACC GAC GAA AAG ATC AGC GTT CAA CAT GAA TGG
        3483        3492        3501        3510        3519        3528

Q   T   G   R   V   K   V   V   V   A   T   I   A   F   G   M   G   I   D   K
CAA ACC GGC CGA GTC AAA GTC GTG GTA GCC ACC ATT GCC TTC GGC ATG GGC ATC GAC AAG
        3543        3552        3561        3570        3579        3588

P   D   V   R   F   V   I   H   Q   H   I   P   K   S   L   E   G   Y   Y   Q
CCT GAC GTC CGC TTT GTT ATC CAC CAG CAC ATC CCC AAG TCG CTC GAA GGT TAC TAT CAA
        3603        3612        3621        3630        3639        3648

E   T   G   R   A   G   R   D   G   K   P   S   D   C   Y   L   Y   F   A   Y
GAA ACC GGC CGC GCC GGA CGT GAC GGC AAG CCA TCG GAC TGC TAC TTG TAC TTT GCC TAT
        3663        3672        3681        3690        3699        3708

G   D   I   Q   S   L   R   R   M   I   A   D   G   E   G   D   Y   A   Q   K
GGC GAC ATT CAA TCC CTA CGT CGT ATG ATC GCC GAC GGC GAA GGT GAC TAC GCG CAA AAG
        3723        3732        3741        3750        3759        3768
```

FIG 5/4

```
E   R   Q   L   Q   M   L   N   R   V   V   S   Y   C   E   S   Q   H   T   C
GAG CGT CAG CTA CAA ATG CTC AAC CGT GTG GTC AGC TAT TGC GAG TCG CAG CAC ACG TGC
        3783        3792        3801        3810        3819        3828

R   R   E   E   V   L   R   Y   F   G   E   E   F   D   Y   R   K   C   R   D
CGG CGC GAA GAA GTG CTC CGC TAC TTT GGC GAG GAG TTT GAC TAC CGG AAG TGT AGA GAC
        3843        3852        3861        3870        3879        3888

G   C   D   N   C   R   N   G   R   I   S   K   S   T   E   M   R   D   F   T
GGA TGC GAT AAC TGC CGG AAC GGA CGC ATC TCG AAG TCG ACG GAG ATG AGG GAT TTT ACG
        3903        3912        3921        3930        3939        3948

E   I   A   F   A   A   I   E   V   V   K   S   Q   Q   P   I   T   L   G   K
GAA ATC GCC TTC GCC GCG ATC GAG GTG GTG AAG AGC CAG CAG CCC ATC ACG CTG GGC AAG
        3963        3972        3981        3990        3999        4008

L   C   D   I   L   M   G   K   R   K   N   E   H   G   G   V   C   H   F   G
CTG TGC GAC ATC CTG ATG GGC AAG AGA AAG AAC GAG CAC GGT GGC GTG TGT CAC TTT GGT
        4023        4032        4041        4050        4059        4068

I   A   K   G   S   T   Q   R   E   L   Q   R   I   V   L   Q   L   N   F   H
ATC GCC AAG GGG AGC ACG CAG AGG GAG CTG CAG AGG ATC GTG CTG CAG CTG AAT TTC CAC
        4083        4092        4101        4110        4119        4128

K   A   L   G   E   D   N   I   M   N   G   A   G   M   P   I   T   Y   Y   I
AAG GCG CTG GGC GAG GAC AAT ATC ATG AAT GGG GCG GGG ATG CCT ATT ACC TAC TAT ATT
        4143        4152        4161        4170        4179        4188

T   G   P   E   A   G   A   Y   L   Y   N   G   K   R   L   M   L   P   V   P
ACC GGC CCT GAA GCT GGT GCT TAC CTC TAC AAT GGC AAG CGG TTG ATG CTG CCA GTT CCC
        4203        4212        4221        4230        4239        4248

S   N   K   S   V   E   P   P   S   R   S   K   Q   R   S   R   R   V   D   E
TCA AAC AAG TCC GTC GAA CCC CCG TCT CGG TCT AAG CAG CGG AGC CGT CGA GTC GAT GAG
        4263        4272        4281        4290        4299        4308

D   M   D   E   Q   E   L   S   T   L   Q   R   P   P   T   S   T   N   V   S
GAT ATG GAT GAG CAA GAA CTT TCC ACC CTG CAA CGA CCG CCA ACA TCA ACA AAT GTC TCT
        4323        4332        4341        4350        4359        4368

S   P   V   R   A   T   K   K   R   S   S   K   K   A   L   P   T   L   I   A
TCA CCC GTT CGA GCC ACC AAG AAA CGA AGT TCC AAA AAG GCT TTA CCG ACC CTC ATC GCC
        4383        4392        4401        4410        4419        4428

D   Y   E   E   P   S   S   D   G   P   H   G   P   L   H   A   N   G   Y   E
GAC TAC GAA GAG CCC AGC TCC GAC GGT CCT CAC GGT CCT CTC CAC GCC AAC GGC TAT GAG
        4443        4452        4461        4470        4479        4488

R   D   N   F   V   V   S   D   N   V   E   P   E   E   E   E   D   A   F   E
CGC GAT AAC TTT GTC GTA TCC GAT AAT GTT GAA CCC GAA GAG GAA GAA GAT GCC TTC GAA
        4503        4512        4521        4530        4539        4548

P   V   R   P   S   R   R   G   P   S   S   R   A   T   R   P   Q   H   R   Q
CCT GTC CGC CCC TCG CGG CGC GGC CCA TCT TCT CGC GCT ACC CGC CCT CAA CAC CGC CAG
        4563        4572        4581        4590        4599        4608

T   T   L   Y   D   T   L   S   H   T   Q   Q   S   Q   T   V   S   Q   H   L
ACC ACC CTT TAT GAC ACC CTC TCC CAC ACC CAA CAA TCC CAA ACC GTC TCC CAA CAC CTC
        4623        4632        4641        4650        4659        4668

A   T   L   G   P   P   I   D   A   R   T   M   H   N   P   R   Y   A   Q   L
GCC ACT TTG GGT CCG CCC ATC GAC GCC CGC ACC ATG CAT AAC CCC CGC TAC GCC CAG CTT
        4683        4692        4701        4710        4719        4728
```

FIG. 5/5

```
D   E   V   H   Q   D   I   V   D   A   F   V   E   E   V   K   V   F   E   E
GAC GAG GTC CAC CAG GAT ATT GTC GAT GCC TTT GTT GAA GAA GTC AAG GTC TTC GAG GAG
        4743        4752        4761        4770        4779        4788

D   F   R   N   R   N   H   M   R   K   P   I   F   T   E   T   Q   Y   R   E
GAC TTT CGC AAC AGG AAC CAC ATG CGC AAA CCC ATC TTT ACC GAG ACG CAG TAC CGT GAG
        4803        4812        4821        4830        4839        4848

M   A   I   R   W   T   R   S   L   D   A   M   R   A   I   P   D   I   N   Q
ATG GCA ATC CGG TGG ACG CGG TCG TTA GAC GCG ATG CGC GCG ATC CCG GAT ATC AAC CAG
        4863        4872        4881        4890        4899        4908

D   K   V   D   R   Y   G   A   K   F   I   P   L   V   E   R   F   W   G   N
GAT AAA GTA GAT CGG TAT GGT GCC AAA TTC ATC CCA CTT GTG GAG CGG TTC TGG GGG AAT
        4923        4932        4941        4950        4959        4968

Y   Q   E   M   M   G   G   G   Y   D   N   P   A   V   A   G   D   E   D   D
TAT CAG GAG ATG ATG GGG GGA GGG TAT GAT AAT CCT GCT GTG GCT GGC GAT GAG GAT GAT
        4983        4992        5001        5010        5019        5028

D   E   G   P   R   R   T   G   N   G   K   G   G   N   K   K   G   G   G   G
GAT GAG GGC CCC AGG AGG ACA GGA AAT GGA AAA GGG GGG AAT AAG AAG GGA GGA GGA GGA
        5043        5052        5061        5070        5079        5088

G   G   G   N   E   V   V   D   L   I   S   S   D   E   D   E   P   P   A   R
GGA GGA GGA AAT GAA GTA GTG GAT TTG ATT AGT AGT GAT GAG GAT GAA CCT CCG GCT CGT
        5103        5112        5121        5130        5139        5148

A   P   S   R   N   A   G   R   G   K   A   Q   S   T   R   G   G   Q   I   Q
GCA CCA TCG CGG AAT GCG GGG CGA GGA AAG GCA CAG TCG ACA CGT GGG GGA CAA ATC CAA
        5163        5172        5181        5190        5199        5208

D   K   G   R   A   V   N   R   R   G   E   P   I   A   E   E   D   E   E   D
GAT AAA GGC CGA GCA GTC AAC CGC CGC GGA GAA CCC ATC GCC GAA GAA GAC GAA GAA GAC
        5223        5232        5241        5250        5259        5268

Y   G   L   S   D   P   D   I   D   A   I   D   P   D   A   I   T   A   S   D
TAC GGG CTA AGC GAC CCC GAT ATC GAC GCC ATC GAT CCA GAC GCC ATC ACC GCC TCC GAC
        5283        5292        5301        5310        5319        5328

N   S   D   E   E   D   D   D   D   D   D   E   D   L   E   S   S   R   Y   F
AAC TCC GAC GAA GAA GAT GAT GAT GAT GAT GAC GAA GAC CTC GAA TCC TCC CGC TAC TTC
        5343        5352        5361        5370        5379        5388

S   G   S   T   G   P   P   V   S   K   A   V   Q   D   A   R   L   R   E   Q
TCC GGC TCA ACA GGC CCG CCC GTC TCC AAA GCC GTG CAG GAT GCT CGA CTC CGT GAA CAA
        5403        5412        5421        5430        5439        5448

L   S   M   Y   A   S   G   G   S   S   S   K   G   S   Y   G   S   G   R   A
CTT TCC ATG TAC GCC TCC GGC GGC AGC TCT TCG AAA GGT AGC TAC GGC TCA GGG CGC GCA
        5463        5472        5481        5490        5499        5508

S   G   G   S   S   S   R   A   S   G   S   G   W   R   G   G   G   A   G   G
TCA GGA GGA TCT TCG TCG AGA GCG TCG GGA TCA GGA TGG AGA GGT GGA GGA GCA GGT GGG
        5523        5532        5541        5550        5559        5568

K   K   Y   Y   R   K   K   R   A   G   S   S   A   A   G   G   G   G   A   G
AAG AAA TAC TAC AGG AAG AAG AGG GCT GGT TCT TCG GCT GCT GGT GGT GGT GGT GCA GGA
        5583        5592        5601        5610        5619        5628

G   G   G   V   T   K   R   K   A   S   G   S   G   A   K   T   A   R   K   R
GGA GGG GGA GTT ACA AAA CGG AAG GCG AGT GGG AGT GGC GCG AAG ACG GCG AGG AAG AGG
        5643        5652        5661        5670        5679        5688
```

FIG. 5/6

```
G   A   S   T   A   P   K   T   T   T   R   G   G   G   S   G   A   G   S   R
GGT GCA TCT ACT GCG CCG AAG ACA ACG ACG AGA GGG GGA GGA TCT GGA GCT GGG TCT AGA
        5703        5712        5721        5730        5739        5748

G   G   G   A   G   G   A   G   G   A   G   A   G   A   G   A   G   G   G   K
GGA GGC GGT GCT GGT GGT GCT GGT GGT GCT GGT GCT GGT GCT GGT GCT GGA GGA GGG AAA
        5763        5772        5781        5790        5799        5808

R   G   G   G   G   G   G   G   M   G   G   I   S   V   M   P   H   .
AGG GGT GGT GGA GGT GGA GGA GGA ATG GGA GGG ATA AGT GTT ATG CCT CAT TAG
        5823        5832        5841        5850        5859
```

FIG. 5/7

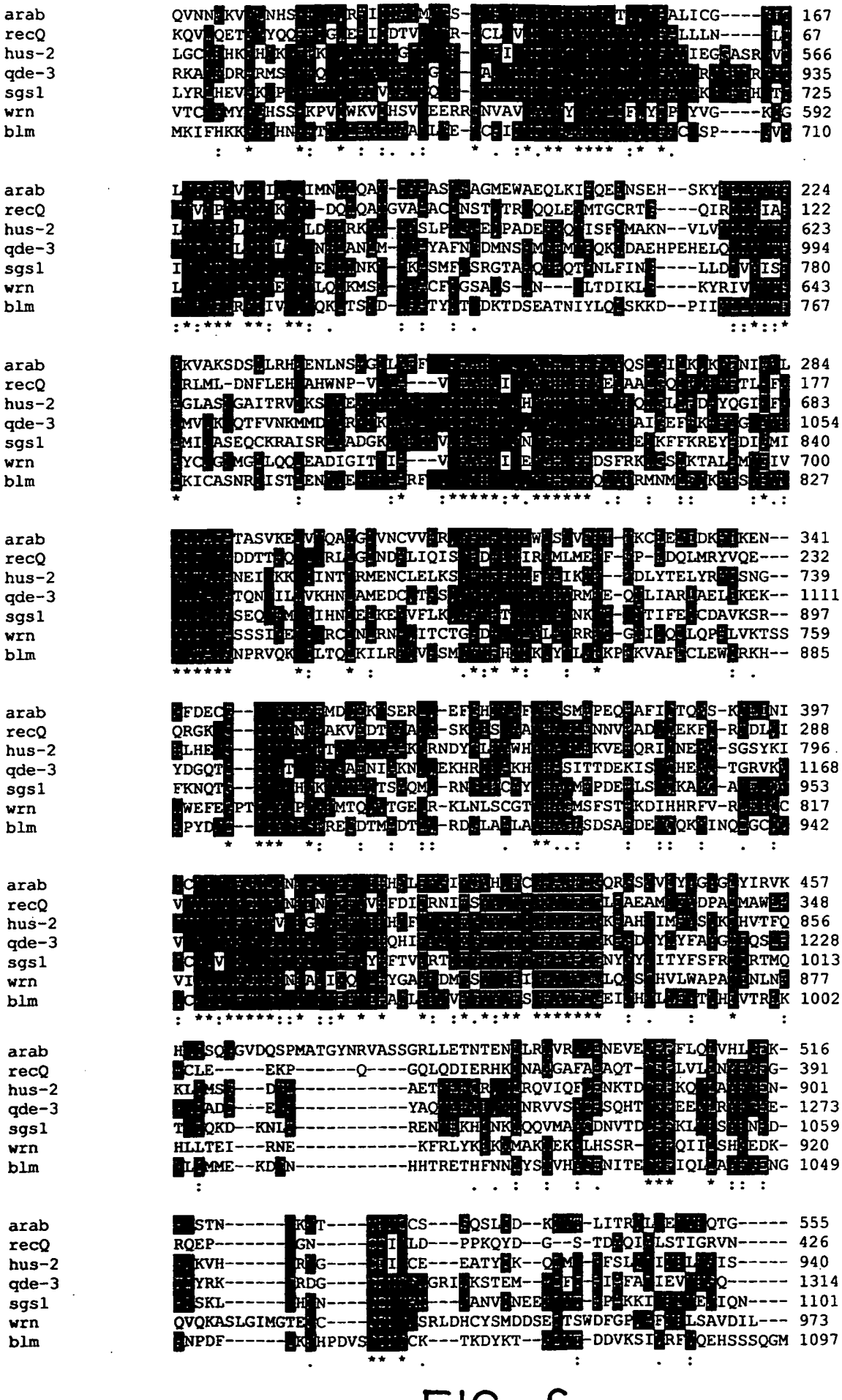


FIG. 6

SEQUENCE LISTING

<110> Università degli studi di Roma La Sapienza
Macino, Giuseppe
Cogoni, Carlo

<120> Isolation and characterization of a N. crassa silencing gene and uses therof

<130> PC

<140>
<141>

<160> 3

<170> PatentIn Ver. 2.1

<210> 1
<211> 6916
<212> DNA
<213> Neurospora crassa

<400> 1
ccaccaccac caccaccacc acaacaacca actcaacaac gaactcaaca accaacccca 60
aacccaacct cgacctcaac ctcaacctca acccttgcga cctcgagata cacaaacaca 120
tcctcgacaa atgacgcccg acccgctaca cgccaacaga ttgccccga ggtaggagca 180
tcgacgcatc aggattcagt tggacttgga gaaggaggag gaggaggaat ggcgaagctc 240
tcagtcaaga acaacctgcc acggccgcac ttggtctcct tgtcgtcgtc aacgacaggc 300
tctgggtctg gttctgcgtc taggtcagct tctgctaagc acggaagtgc cggttccagt 360
acctttgatc atgaacaaca tcaacaacat caacaacaac aacaacaaaa gcgccagcgg 420
tcgcaatcag aagcacgaca acagcagcag caacagcaac agcaacagca acagcaacaa 480
caacaacaac aagcacagca ccatgcacat tctacatatg cacaaagacc ccaacccacc 540
ccccaacaac gaccacccca aaacctactg acacctgctt caaccactgg tgccagcgtc 600
ggcccgctcc aacgcgcata ctcggtttca ttagctgcga gacagtcccc ctcgacaaac 660
ttggtccgtc caaagaccga ctcgccagct ccccacactt tacacctcaa gaacaagaag 720
aacctccgtc accccgcccc cacgcccgac agtccgatcg tagacgacga tattttctcc 780
gacgccgtcg atcttaccga agaactcgat catgaccatg atctcaacgg caaagacaaa 840
gacaacaccg acaacgacaa cacagtcgct tccagttcgc taatagggtt cggcgatgac 900
aagttactgt ggcgagagga ctttgctgag cgtgcagagc ccgaacatga aagaggtggg 960
agcaggcctc gccaggtcaa gaaacggaag atatcgaatg actacattat gaaggatgag 1020
gatgtctcgc tttttgatga tgatggcgag gaggacgagt ttatggatat caatgagcta 1080
gttcaggggg atcgggaaag tactccgaag ccaaaggcta catcgaggtc tgtctcgacg 1140
aggctgccgc ctacagtatc gctgcaacgg ggtcggtctc ctaagaggaa ggaggcttca 1200
gttgaaaagc gcacaacgga aaaccagcaa caggctgaca gagaagacga accgtcgttt 1260
atgtcaagtc cagatgtcga caactcccgc aagcgaaagt cttctggatc gcccacaggt 1320
ttaacgacgc caagacccca gcagaagcaa acggaagagg tcccaggtac gaccaccgcc 1380
aagaagccac ggcgcagtga agtgatggac tcggaggacg aggcattcac tcctctttct 1440

gctgggtcgc tgcctgggag tgcggagttc ttcagaagcg gtgggaccac cacacgggaa 1500
ttgggtttgg acgaagacac ggttatggac acgcctagta ggccaccggt cgagtccact 1560
ttgccaactc tcgagtctgt ggaaagtcga ccacccccc tgccgcccat ggatctacca 1620
tcacagcgaa aaccgctaga gccgttgaac actccgcgca accagctgct tgagtcggtc 1680
gaaaggccaa cacagcagcc gtcggtgggg ccgagttttg cacagagtag cacactcgcc 1740
gaaagctccc tgccgccgtc aatgccgccg ccaagtgaag acccccctcaa caccagggag 1800
aacagcaacc ttgaggagtt cgactacaag ctttacaaac ccctgctaga tcttttcgtc 1860
aacgcacccg caatcttgga aagagaactg agcgccgtta atgacgagct tcaggagaac 1920
atgatcaagc tgcgggactg tctgcgcctg cccagggaag aaagagacag ggcacgcgaa 1980
gaggtgaaga aggaaaagga aatgctcaag cgacgggaca ttgcgctcag agccctccag 2040
gacgaacaca agttgtacgt caagaaacgc aaagagcata atttgatcaa cgaggaaatc 2100
gttcgcgctt atgctgaaga agacgatgag tacgaggatc agttaatggc gcagctggac 2160
aagttggatg atgaggttga ggctatcgta aagagtctga caaggcttat tgtggcggcg 2220
gggatcacgg agaagagctt tgacctaaag aaggaggagg aagaggagga ggagaagccg 2280
atcatcatag cgactccgac accttcgacg aggaccgagg ccccggttct gccgacgacc 2340
gagtatcata attcccagca ggtcatattg cagactcaac atcctgctgc gcagcaggtt 2400
tctcaccggg tgccaccacc tccgacaccg agttttcaaa cggcgcgcca gactccggtg 2460
tcatatcaga gcagaccgac caacaactcc tttcctgata tctcggcgga agaagccatg 2520
atgttcgata aagaagaccc cttcatggaa caacagcacg ccccggcctc tgctcccttc 2580
caggccaccc ttccccagcg caacagccct ttcaaaaccg ccccgttcaa gccagtccac 2640
ggccacgatt actttgacga tgaagacgac gatgccgacc tcctggcagc agtagacagc 2700
gccgagacgt atacttctac ggccgccacc accaccacca acaacaacaa tcacttacga 2760
tcacaatcgg tgatgtcaac atccacggcg accacgatca aaccgaggaa acgcaacgaa 2820
aatgccaatg ccaagaagcc caagtccgta catgcaaagt tatcgatgcc gcccgaaaag 2880
atgaagtatg cgtggtcgaa tgatgtgagg aaggctctca aggataggtt tcggatgtcg 2940
gggttcagac agaatcagtt ggaggctatt aatgctactt tgggtggtaa ggtgagttct 3000
ctgtccttta cctatctggg agagaccaag aaggagagag agagagagag gaggggaaga 3060
cgaaaatgga ctttgctgac tctagaaagg atgcctttgt gttgatgccg actggtggtg 3120
gaaagtctct gtgctatcag ttgccggctg tagtcaggag cggcaagacg cgtggtatca 3180
cagtcgtcat ctcccctctg ctaagtctga tgctggatca agtcaaccat ttggcaaacc 3240
tgatgatcca agcttacgct ttcaacggag acatgaactc agaaatgcgc cgaatggtgt 3300
ttcagaagct tgatgctgag catcctgagc atgagctcca actgctctat gtcaccccgg 3360
agatggtgag caagaaccag acattcgtca acaagatgat ggacctctac cggaggaaaa 3420
agctggctag aattgttatc gacgaggctc actgcgtcag tcaatggggc catgacttcc 3480
gacccgatta caaagctata ggagagtttc gtaagaggtt tcccggagtt ccggtcatgg 3540
ctttgacagc gacggcaaca cagaacgtca tcttggatgt caagcataac ctggcaatgg 3600
aggactgcca gactttctcc cagagcttta atcggccgaa cctctactat gaggtcagga 3660
tgaaggagca gaatctgatt gcccgcatcg cagagttgat caaggagaag tatgacggcc 3720
agacgggtat catctacaca ttatcaagaa agagtgccga gaacatcgcc aaaaatctcc 3780
aggaaaaaca ccgcatcaaa gcaaagcact accatgcctc catcaccacc gacgaaaaga 3840
tcagcgttca acatgaatgg caaaccggcc gagtcaaagt cgtggtagcc accattgcct 3900
tcggcatggg catcgacaag cctgacgtcc gctttgttat ccaccagcac atccccaagt 3960
cgctcgaagg ttactatcaa gaaaccggcc gcgccggacg tgacggcaag ccatcggact 4020
gctacttgta ctttgcctat ggcgacattc aatccctacg tcgtatgatc gccgacggcg 4080
aaggtgacta cgcgcaaaag gagcgtcagc tacaaatgct caaccgtgtg gtcagctatt 4140
gcgagtcgca gcacacgtgc cggcgcgaag aagtgctccg ctactttggc gaggagtttg 4200
actaccggaa gtgtagagac ggatgcgata actgccggaa cggacgcatc tcgaagtcga 4260
cggagatgag ggattttacg gaaatcgcct tcgccgcgat cgaggtggtg aagagccagc 4320

agcccatcac gctgggcaag ctgtgcgaca tcctgatggg caagagaaag aacgagcacg 4380
gtggcgtgtg tcactttggt atcgccaagg ggagcacgca gagggagctg cagaggatcg 4440
tgctgcagct gaatttccac aaggcgctgg gcgaggacaa tatcatgaat ggggcgggga 4500
tgcctattac ctactatatt gtgagtgctg tcccggttgg tcttgcatat ctggctttgt 4560
tgctttgcta acacagcagc tcgtacagac cggccctgaa gctggtgctt acctctacaa 4620
tggcaagcgg ttgatgctgc cagttccctc aaacaagtcc gtcgaacccc cgtctcggtc 4680
taagcagcgg agccgtcgag tcgatgagga tatggatgag caagaacttt ccaccctgca 4740
acgaccgcca acatcaacaa atgtctcttc acccgttcga gccaccaaga aacgaagttc 4800
caaaaaggct ttaccgaccc tcatcgccga ctacgaagag cccagctccg acggtcctca 4860
cggtcctctc cacgccaacg gctatgagcg cgataacttt gtcgtatccg ataatgttga 4920
acccgaagag gaagaagatg ccttcgaacc tgtccgcccc tcgcggcgcg gcccatcttc 4980
tcgcgctacc cgccctcaac accgccagac cacccttttat gacaccctct cccacaccca 5040
acaatcccaa accgtctccc aacacctcgc cactttgggt ccgcccatcg acgcccgcac 5100
catgcataac ccccgctacg cccagcttga cgaggtccac caggatattg tcgatgcctt 5160
tgttgaagaa gtcaaggtct tcgaggagga ctttcgcaac aggaaccaca tgcgcaaacc 5220
catctttacc gagacgcagt accgtgagat ggcaatccgg tggacgcggt cgttagacgc 5280
gatgcgcgcg atcccggata tcaaccagga taaagtagat cggtatggtg ccaaattcat 5340
cccacttgtg gagcggttct gggggaatta tcaggagatg atgggggggag ggtatgataa 5400
tcctgctgtg gctggcgatg aggatgatga tgagggcccc aggaggacag gaaatggaaa 5460
agggggggaat aagaagggag gaggaggagg aggaggaaat gaagtagtgg atttgattag 5520
tagtgatgag gatgaacctc cggctcgtgc accatcgcgg aatgcggggc gaggaaaggc 5580
acagtcgaca cgtgggggac aaatccaaga taaaggccga gcagtcaacc gccgcggaga 5640
acccatcgcc gaagaagacg aagaagacta cgggctaagc gaccccgata tcgacgccat 5700
cgatccagac gccatcaccg cctccgacaa ctccgacgaa gaagatgatg atgatgatga 5760
cgaagacctc gaatcctccc gctacttctc cggctcaaca ggcccgcccg tctccaaagc 5820
cgtgcaggat gctcgactcc gtgaacaact ttccatgtac gcctccggcg gcagctcttc 5880
gaaaggtagc tacggctcag ggcgcgcatc aggaggatct tcgtcgagag cgtcgggatc 5940
aggatggaga ggtggaggag caggtgggaa gaaatactac aggaagaaga gggctggttc 6000
ttcggctgct ggtggtggtg gtgcaggagg agggggagtt acaaaacgga aggcgagtgg 6060
gagtggcgcg aagacggcga ggaagagggg tgcatctact gcgccgaaga caacgacgag 6120
agggggagga tctggagctg ggtctagagg aggcggtgct ggtggtgctg gtggtgctgg 6180
tgctggtgct ggtgctggag gagggaaaag gggtggtgga ggtggaggag gaatgggagg 6240
gataagtgtt atgcctcatt agctatttta tagcatatcg catttataca gtgtcttatg 6300
gaagggagga ggagaagaag aaggataagc tggcataagc ttgaaccggc caggccaaaa 6360
tggccagaga gctcaccggg caatcgagct tgaaatgagc ttgacatatt aggtattccc 6420
gagaatatag cgggattaca aggcacttac tttaccaagt cgaaagggac gagccaaatc 6480
tatggtactc gccagttgcg caacgttgag ttttatcatt cgtggagttt tcatcgtgga 6540
gtttttatta tcaactattc gttgtatagt tttcgttgta gatgttagtt ccggacgatc 6600
aaaaggggaa gtgtggaaca gagaagtcga aaggacaagc caaaatgaca tggcagtgtc 6660
cagtcagata ccctccagac aaaaccagac accaataaca aacccttcaa ccataacacc 6720
agcaaagcca atccttaggt acctacctag ggtagggtag gtccaggaat gtcttcccca 6780
aaggtacctc tacttattca tgttacgctc catcagtccc atcgcttagc atcgctgccc 6840
ggttacctat ctctacctct acctctacct ctacctctac ctctacctct acctctatct 6900
ctacctctac ctctac 6916

<210> 2
<211> 5868

<212> DNA
<213> Neurospora crassa

<220>
<221> CDS
<222> (1)..(5868)

<400> 2

```
atg gcg aag ctc tca gtc aag aac aac ctg cca cgg ccg cac ttg gtc   48
Met Ala Lys Leu Ser Val Lys Asn Asn Leu Pro Arg Pro His Leu Val
  1               5                  10                  15

tcc ttg tcg tcg tca acg aca ggc tct ggg tct ggt tct gcg tct agg   96
Ser Leu Ser Ser Ser Thr Thr Gly Ser Gly Ser Gly Ser Ala Ser Arg
             20                  25                  30

tca gct tct gct aag cac gga agt gcc ggt tcc agt acc ttt gat cat   144
Ser Ala Ser Ala Lys His Gly Ser Ala Gly Ser Ser Thr Phe Asp His
         35                  40                  45

gaa caa cat caa caa cat caa caa caa caa caa caa aag cgc cag cgg   192
Glu Gln His Gln Gln His Gln Gln Gln Gln Gln Gln Lys Arg Gln Arg
     50                  55                  60

tcg caa tca gaa gca cga caa cag cag cag caa cag caa cag caa cag   240
Ser Gln Ser Glu Ala Arg Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
 65                  70                  75                  80

caa cag caa caa caa caa caa caa gca cag cac cat gca cat tct aca   288
Gln Gln Gln Gln Gln Gln Gln Gln Ala Gln His His Ala His Ser Thr
                 85                  90                  95

tat gca caa aga ccc caa ccc acc ccc caa caa cga cca ccc caa aac   336
Tyr Ala Gln Arg Pro Gln Pro Thr Pro Gln Gln Arg Pro Pro Gln Asn
            100                 105                 110

cta ctg aca cct gct tca acc act ggt gcc agc gtc ggc ccg ctc caa   384
Leu Leu Thr Pro Ala Ser Thr Thr Gly Ala Ser Val Gly Pro Leu Gln
        115                 120                 125

cgc gca tac tcg gtt tca tta gct gcg aga cag tcc ccc tcg aca aac   432
Arg Ala Tyr Ser Val Ser Leu Ala Ala Arg Gln Ser Pro Ser Thr Asn
    130                 135                 140

ttg gtc cgt cca aag acc gac tcg cca gct ccc cac act tta cac ctc   480
Leu Val Arg Pro Lys Thr Asp Ser Pro Ala Pro His Thr Leu His Leu
145                 150                 155                 160
```

```
aag aac aag aag aac ctc cgt cac ccc gcc ccc acg ccc gac agt ccg   528
Lys Asn Lys Lys Asn Leu Arg His Pro Ala Pro Thr Pro Asp Ser Pro
                165                 170                 175

atc gta gac gac gat att ttc tcc gac gcc gtc gat ctt acc gaa gaa   576
Ile Val Asp Asp Asp Ile Phe Ser Asp Ala Val Asp Leu Thr Glu Glu
            180                 185                 190

ctc gat cat gac cat gat ctc aac ggc aaa gac aaa gac aac acc gac   624
Leu Asp His Asp His Asp Leu Asn Gly Lys Asp Lys Asp Asn Thr Asp
        195                 200                 205

aac gac aac aca gtc gct tcc agt tcg cta ata ggg ttc ggc gat gac   672
Asn Asp Asn Thr Val Ala Ser Ser Ser Leu Ile Gly Phe Gly Asp Asp
    210                 215                 220

aag tta ctg tgg cga gag gac ttt gct gag cgt gca gag ccc gaa cat   720
Lys Leu Leu Trp Arg Glu Asp Phe Ala Glu Arg Ala Glu Pro Glu His
225                 230                 235                 240

gaa aga ggt ggg agc agg cct cgc cag gtc aag aaa cgg aag ata tcg   768
Glu Arg Gly Gly Ser Arg Pro Arg Gln Val Lys Lys Arg Lys Ile Ser
                245                 250                 255

aat gac tac att atg aag gat gag gat gtc tcg ctt ttt gat gat gat   816
Asn Asp Tyr Ile Met Lys Asp Glu Asp Val Ser Leu Phe Asp Asp Asp
            260                 265                 270

ggc gag gag gac gag ttt atg gat atc aat gag cta gtt cag ggg gat   864
Gly Glu Glu Asp Glu Phe Met Asp Ile Asn Glu Leu Val Gln Gly Asp
        275                 280                 285

cgg gaa agt act ccg aag cca aag gct aca tcg agg tct gtc tcg acg   912
Arg Glu Ser Thr Pro Lys Pro Lys Ala Thr Ser Arg Ser Val Ser Thr
    290                 295                 300

agg ctg ccg cct aca gta tcg ctg caa cgg ggt cgg tct cct aag agg   960
Arg Leu Pro Pro Thr Val Ser Leu Gln Arg Gly Arg Ser Pro Lys Arg
305                 310                 315                 320

aag gag gct tca gtt gaa aag cgc aca acg gaa aac cag caa cag gct  1008
Lys Glu Ala Ser Val Glu Lys Arg Thr Thr Glu Asn Gln Gln Gln Ala
                325                 330                 335

gac aga gaa gac gaa ccg tcg ttt atg tca agt cca gat gtc gac aac  1056
Asp Arg Glu Asp Glu Pro Ser Phe Met Ser Ser Pro Asp Val Asp Asn
            340                 345                 350
```

```
tcc cgc aag cga aag tct tct gga tcg ccc aca ggt tta acg acg cca   1104
Ser Arg Lys Arg Lys Ser Ser Gly Ser Pro Thr Gly Leu Thr Thr Pro
        355                 360                 365

aga ccc cag cag aag caa acg gaa gag gtc cca ggt acg acc acc gcc   1152
Arg Pro Gln Gln Lys Gln Thr Glu Glu Val Pro Gly Thr Thr Thr Ala
    370                 375                 380

aag aag cca cgg cgc agt gaa gtg atg gac tcg gag gac gag gca ttc   1200
Lys Lys Pro Arg Arg Ser Glu Val Met Asp Ser Glu Asp Glu Ala Phe
385                 390                 395                 400

act cct ctt tct gct ggg tcg ctg cct ggg agt gcg gag ttc ttc aga   1248
Thr Pro Leu Ser Ala Gly Ser Leu Pro Gly Ser Ala Glu Phe Phe Arg
                405                 410                 415

agc ggt ggg acc acc aca cgg gaa ttg ggt ttg gac gaa gac acg gtt   1296
Ser Gly Gly Thr Thr Thr Arg Glu Leu Gly Leu Asp Glu Asp Thr Val
            420                 425                 430

atg gac acg cct agt agg cca ccg gtc gag tcc act ttg cca act ctc   1344
Met Asp Thr Pro Ser Arg Pro Pro Val Glu Ser Thr Leu Pro Thr Leu
        435                 440                 445

gag tct gtg gaa agt cga cca ccc ccc ctg ccg ccc atg gat cta cca   1392
Glu Ser Val Glu Ser Arg Pro Pro Pro Leu Pro Pro Met Asp Leu Pro
    450                 455                 460

tca cag cga aaa ccg cta gag ccg ttg aac act ccg cgc aac cag ctg   1440
Ser Gln Arg Lys Pro Leu Glu Pro Leu Asn Thr Pro Arg Asn Gln Leu
465                 470                 475                 480

ctt gag tcg gtc gaa agg cca aca cag cag ccg tcg gtg ggg ccg agt   1488
Leu Glu Ser Val Glu Arg Pro Thr Gln Gln Pro Ser Val Gly Pro Ser
                485                 490                 495

ttt gca cag agt agc aca ctc gcc gaa agc tcc ctg ccg ccg tca atg   1536
Phe Ala Gln Ser Ser Thr Leu Ala Glu Ser Ser Leu Pro Pro Ser Met
            500                 505                 510

ccg ccg cca agt gaa gac ccc ctc aac acc agg gag aac agc aac ctt   1584
Pro Pro Pro Ser Glu Asp Pro Leu Asn Thr Arg Glu Asn Ser Asn Leu
        515                 520                 525

gag gag ttc gac tac aag ctt tac aaa ccc ctg cta gat ctt ttc gtc   1632
Glu Glu Phe Asp Tyr Lys Leu Tyr Lys Pro Leu Leu Asp Leu Phe Val
    530                 535                 540
```

```
aac gca ccc gca atc ttg gaa aga gaa ctg agc gcc gtt aat gac gag   1680
Asn Ala Pro Ala Ile Leu Glu Arg Glu Leu Ser Ala Val Asn Asp Glu
545                 550                 555                 560

ctt cag gag aac atg atc aag ctg cgg gac tgt ctg cgc ctg ccc agg   1728
Leu Gln Glu Asn Met Ile Lys Leu Arg Asp Cys Leu Arg Leu Pro Arg
                565                 570                 575

gaa gaa aga gac agg gca cgc gaa gag gtg aag aag gaa aag gaa atg   1776
Glu Glu Arg Asp Arg Ala Arg Glu Glu Val Lys Lys Glu Lys Glu Met
            580                 585                 590

ctc aag cga cgg gac att gcg ctc aga gcc ctc cag gac gaa cac aag   1824
Leu Lys Arg Arg Asp Ile Ala Leu Arg Ala Leu Gln Asp Glu His Lys
        595                 600                 605

ttg tac gtc aag aaa cgc aaa gag cat aat ttg atc aac gag gaa atc   1872
Leu Tyr Val Lys Lys Arg Lys Glu His Asn Leu Ile Asn Glu Glu Ile
    610                 615                 620

gtt cgc gct tat gct gaa gaa gac gat gag tac gag gat cag tta atg   1920
Val Arg Ala Tyr Ala Glu Glu Asp Asp Glu Tyr Glu Asp Gln Leu Met
625                 630                 635                 640

gcg cag ctg gac aag ttg gat gat gag gtt gag gct atc gta aag agt   1968
Ala Gln Leu Asp Lys Leu Asp Asp Glu Val Glu Ala Ile Val Lys Ser
                645                 650                 655

ctg aca agg ctt att gtg gcg gcg ggg atc acg gag aag agc ttt gac   2016
Leu Thr Arg Leu Ile Val Ala Ala Gly Ile Thr Glu Lys Ser Phe Asp
            660                 665                 670

cta aag aag gag gag gaa gag gag gag gag aag ccg atc atc ata gcg   2064
Leu Lys Lys Glu Glu Glu Glu Glu Glu Glu Lys Pro Ile Ile Ile Ala
        675                 680                 685

act ccg aca cct tcg acg agg acc gag gcc ccg gtt ctg ccg acg acc   2112
Thr Pro Thr Pro Ser Thr Arg Thr Glu Ala Pro Val Leu Pro Thr Thr
    690                 695                 700

gag tat cat aat tcc cag cag gtc ata ttg cag act caa cat cct gct   2160
Glu Tyr His Asn Ser Gln Gln Val Ile Leu Gln Thr Gln His Pro Ala
705                 710                 715                 720

gcg cag cag gtt tct cac cgg gtg cca cca cct ccg aca ccg agt ttt   2208
Ala Gln Gln Val Ser His Arg Val Pro Pro Pro Pro Thr Pro Ser Phe
                725                 730                 735
```

```
caa acg gcg cgc cag act ccg gtg tca tat cag agc aga ccg acc aac   2256
Gln Thr Ala Arg Gln Thr Pro Val Ser Tyr Gln Ser Arg Pro Thr Asn
            740                 745                 750

aac tcc ttt cct gat atc tcg gcg gaa gaa gcc atg atg ttc gat aaa   2304
Asn Ser Phe Pro Asp Ile Ser Ala Glu Glu Ala Met Met Phe Asp Lys
        755                 760                 765

gaa gac ccc ttc atg gaa caa cag cac gcc ccg gcc tct gct ccc ttc   2352
Glu Asp Pro Phe Met Glu Gln Gln His Ala Pro Ala Ser Ala Pro Phe
    770                 775                 780

cag gcc acc ctt ccc cag cgc aac agc cct ttc aaa acc gcc ccg ttc   2400
Gln Ala Thr Leu Pro Gln Arg Asn Ser Pro Phe Lys Thr Ala Pro Phe
785                 790                 795                 800

aag cca gtc cac ggc cac gat tac ttt gac gat gaa gac gac gat gcc   2448
Lys Pro Val His Gly His Asp Tyr Phe Asp Asp Glu Asp Asp Asp Ala
                805                 810                 815

gac ctc ctg gca gca gta gac agc gcc gag acg tat act tct acg gcc   2496
Asp Leu Leu Ala Ala Val Asp Ser Ala Glu Thr Tyr Thr Ser Thr Ala
            820                 825                 830

gcc acc acc acc acc aac aac aac aat cac tta cga tca caa tcg gtg   2544
Ala Thr Thr Thr Thr Asn Asn Asn Asn His Leu Arg Ser Gln Ser Val
        835                 840                 845

atg tca aca tcc acg gcg acc acg atc aaa ccg agg aaa cgc aac gaa   2592
Met Ser Thr Ser Thr Ala Thr Thr Ile Lys Pro Arg Lys Arg Asn Glu
    850                 855                 860

aat gcc aat gcc aag aag ccc aag tcc gta cat gca aag tta tcg atg   2640
Asn Ala Asn Ala Lys Lys Pro Lys Ser Val His Ala Lys Leu Ser Met
865                 870                 875                 880

ccg ccc gaa aag atg aag tat gcg tgg tcg aat gat gtg agg aag gct   2688
Pro Pro Glu Lys Met Lys Tyr Ala Trp Ser Asn Asp Val Arg Lys Ala
                885                 890                 895

ctc aag gat agg ttt cgg atg tcg ggg ttc aga cag aat cag ttg gag   2736
Leu Lys Asp Arg Phe Arg Met Ser Gly Phe Arg Gln Asn Gln Leu Glu
            900                 905                 910

gct att aat gct act ttg ggt ggt aag gat gcc ttt gtg ttg atg ccg   2784
Ala Ile Asn Ala Thr Leu Gly Gly Lys Asp Ala Phe Val Leu Met Pro
        915                 920                 925
```

act ggt ggt gga aag tct ctg tgc tat cag ttg ccg gct gta gtc agg 2832
Thr Gly Gly Gly Lys Ser Leu Cys Tyr Gln Leu Pro Ala Val Val Arg
930 935 940

agc ggc aag acg cgt ggt atc aca gtc gtc atc tcc cct ctg cta agt 2880
Ser Gly Lys Thr Arg Gly Ile Thr Val Val Ile Ser Pro Leu Leu Ser
945 950 955 960

ctg atg ctg gat caa gtc aac cat ttg gca aac ctg atg atc caa gct 2928
Leu Met Leu Asp Gln Val Asn His Leu Ala Asn Leu Met Ile Gln Ala
965 970 975

tac gct ttc aac gga gac atg aac tca gaa atg cgc cga atg gtg ttt 2976
Tyr Ala Phe Asn Gly Asp Met Asn Ser Glu Met Arg Arg Met Val Phe
980 985 990

cag aag ctt gat gct gag cat cct gag cat gag ctc caa ctg ctc tat 3024
Gln Lys Leu Asp Ala Glu His Pro Glu His Glu Leu Gln Leu Leu Tyr
995 1000 1005

gtc acc ccg gag atg gtg agc aag aac cag aca ttc gtc aac aag atg 3072
Val Thr Pro Glu Met Val Ser Lys Asn Gln Thr Phe Val Asn Lys Met
1010 1015 1020

atg gac ctc tac cgg agg aaa aag ctg gct aga att gtt atc gac gag 3120
Met Asp Leu Tyr Arg Arg Lys Lys Leu Ala Arg Ile Val Ile Asp Glu
1025 1030 1035 1040

gct cac tgc gtc agt caa tgg ggc cat gac ttc cga ccc gat tac aaa 3168
Ala His Cys Val Ser Gln Trp Gly His Asp Phe Arg Pro Asp Tyr Lys
1045 1050 1055

gct ata gga gag ttt cgt aag agg ttt ccc gga gtt ccg gtc atg gct 3216
Ala Ile Gly Glu Phe Arg Lys Arg Phe Pro Gly Val Pro Val Met Ala
1060 1065 1070

ttg aca gcg acg gca aca cag aac gtc atc ttg gat gtc aag cat aac 3264
Leu Thr Ala Thr Ala Thr Gln Asn Val Ile Leu Asp Val Lys His Asn
1075 1080 1085

ctg gca atg gag gac tgc cag act ttc tcc cag agc ttt aat cgg ccg 3312
Leu Ala Met Glu Asp Cys Gln Thr Phe Ser Gln Ser Phe Asn Arg Pro
1090 1095 1100

aac ctc tac tat gag gtc agg atg aag gag cag aat ctg att gcc cgc 3360
Asn Leu Tyr Tyr Glu Val Arg Met Lys Glu Gln Asn Leu Ile Ala Arg
1105 1110 1115 1120

atc gca gag ttg atc aag gag aag tat gac ggc cag acg ggt atc atc 3408
Ile Ala Glu Leu Ile Lys Glu Lys Tyr Asp Gly Gln Thr Gly Ile Ile
1125 1130 1135

tac aca tta tca aga aag agt gcc gag aac atc gcc aaa aat ctc cag 3456
Tyr Thr Leu Ser Arg Lys Ser Ala Glu Asn Ile Ala Lys Asn Leu Gln
1140 1145 1150

gaa aaa cac cgc atc aaa gca aag cac tac cat gcc tcc atc acc acc 3504
Glu Lys His Arg Ile Lys Ala Lys His Tyr His Ala Ser Ile Thr Thr
1155 1160 1165

gac gaa aag atc agc gtt caa cat gaa tgg caa acc ggc cga gtc aaa 3552
Asp Glu Lys Ile Ser Val Gln His Glu Trp Gln Thr Gly Arg Val Lys
1170 1175 1180

gtc gtg gta gcc acc att gcc ttc ggc atg ggc atc gac aag cct gac 3600
Val Val Val Ala Thr Ile Ala Phe Gly Met Gly Ile Asp Lys Pro Asp
1185 1190 1195 1200

gtc cgc ttt gtt atc cac cag cac atc ccc aag tcg ctc gaa ggt tac 3648
Val Arg Phe Val Ile His Gln His Ile Pro Lys Ser Leu Glu Gly Tyr
1205 1210 1215

tat caa gaa acc ggc cgc gcc gga cgt gac ggc aag cca tcg gac tgc 3696
Tyr Gln Glu Thr Gly Arg Ala Gly Arg Asp Gly Lys Pro Ser Asp Cys
1220 1225 1230

tac ttg tac ttt gcc tat ggc gac att caa tcc cta cgt cgt atg atc 3744
Tyr Leu Tyr Phe Ala Tyr Gly Asp Ile Gln Ser Leu Arg Arg Met Ile
1235 1240 1245

gcc gac ggc gaa ggt gac tac gcg caa aag gag cgt cag cta caa atg 3792
Ala Asp Gly Glu Gly Asp Tyr Ala Gln Lys Glu Arg Gln Leu Gln Met
1250 1255 1260

ctc aac cgt gtg gtc agc tat tgc gag tcg cag cac acg tgc cgg cgc 3840
Leu Asn Arg Val Val Ser Tyr Cys Glu Ser Gln His Thr Cys Arg Arg
1265 1270 1275 1280

gaa gaa gtg ctc cgc tac ttt ggc gag gag ttt gac tac cgg aag tgt 3888
Glu Glu Val Leu Arg Tyr Phe Gly Glu Glu Phe Asp Tyr Arg Lys Cys
1285 1290 1295

aga gac gga tgc gat aac tgc cgg aac gga cgc atc tcg aag tcg acg 3936
Arg Asp Gly Cys Asp Asn Cys Arg Asn Gly Arg Ile Ser Lys Ser Thr
1300 1305 1310

```
gag atg agg gat ttt acg gaa atc gcc ttc gcc gcg atc gag gtg gtg   3984
Glu Met Arg Asp Phe Thr Glu Ile Ala Phe Ala Ala Ile Glu Val Val
       1315                1320                1325

aag agc cag cag ccc atc acg ctg ggc aag ctg tgc gac atc ctg atg   4032
Lys Ser Gln Gln Pro Ile Thr Leu Gly Lys Leu Cys Asp Ile Leu Met
   1330                1335                1340

ggc aag aga aag aac gag cac ggt ggc gtg tgt cac ttt ggt atc gcc   4080
Gly Lys Arg Lys Asn Glu His Gly Gly Val Cys His Phe Gly Ile Ala
1345                1350                1355                1360

aag ggg agc acg cag agg gag ctg cag agg atc gtg ctg cag ctg aat   4128
Lys Gly Ser Thr Gln Arg Glu Leu Gln Arg Ile Val Leu Gln Leu Asn
               1365                1370                1375

ttc cac aag gcg ctg ggc gag gac aat atc atg aat ggg gcg ggg atg   4176
Phe His Lys Ala Leu Gly Glu Asp Asn Ile Met Asn Gly Ala Gly Met
           1380                1385                1390

cct att acc tac tat att acc ggc cct gaa gct ggt gct tac ctc tac   4224
Pro Ile Thr Tyr Tyr Ile Thr Gly Pro Glu Ala Gly Ala Tyr Leu Tyr
       1395                1400                1405

aat ggc aag cgg ttg atg ctg cca gtt ccc tca aac aag tcc gtc gaa   4272
Asn Gly Lys Arg Leu Met Leu Pro Val Pro Ser Asn Lys Ser Val Glu
   1410                1415                1420

ccc ccg tct cgg tct aag cag cgg agc cgt cga gtc gat gag gat atg   4320
Pro Pro Ser Arg Ser Lys Gln Arg Ser Arg Arg Val Asp Glu Asp Met
1425                1430                1435                1440

gat gag caa gaa ctt tcc acc ctg caa cga ccg cca aca tca aca aat   4368
Asp Glu Gln Glu Leu Ser Thr Leu Gln Arg Pro Pro Thr Ser Thr Asn
               1445                1450                1455

gtc tct tca ccc gtt cga gcc acc aag aaa cga agt tcc aaa aag gct   4416
Val Ser Ser Pro Val Arg Ala Thr Lys Lys Arg Ser Ser Lys Lys Ala
           1460                1465                1470

tta ccg acc ctc atc gcc gac tac gaa gag ccc agc tcc gac ggt cct   4464
Leu Pro Thr Leu Ile Ala Asp Tyr Glu Glu Pro Ser Ser Asp Gly Pro
       1475                1480                1485

cac ggt cct ctc cac gcc aac ggc tat gag cgc gat aac ttt gtc gta   4512
His Gly Pro Leu His Ala Asn Gly Tyr Glu Arg Asp Asn Phe Val Val
   1490                1495                1500
```

```
tcc gat aat gtt gaa ccc gaa gag gaa gaa gat gcc ttc gaa cct gtc   4560
Ser Asp Asn Val Glu Pro Glu Glu Glu Glu Asp Ala Phe Glu Pro Val
1505                1510                1515                1520

cgc ccc tcg cgg cgc ggc cca tct tct cgc gct acc cgc cct caa cac   4608
Arg Pro Ser Arg Arg Gly Pro Ser Ser Arg Ala Thr Arg Pro Gln His
                1525                1530                1535

cgc cag acc acc ctt tat gac acc ctc tcc cac acc caa caa tcc caa   4656
Arg Gln Thr Thr Leu Tyr Asp Thr Leu Ser His Thr Gln Gln Ser Gln
            1540                1545                1550

acc gtc tcc caa cac ctc gcc act ttg ggt ccg ccc atc gac gcc cgc   4704
Thr Val Ser Gln His Leu Ala Thr Leu Gly Pro Pro Ile Asp Ala Arg
        1555                1560                1565

acc atg cat aac ccc cgc tac gcc cag ctt gac gag gtc cac cag gat   4752
Thr Met His Asn Pro Arg Tyr Ala Gln Leu Asp Glu Val His Gln Asp
    1570                1575                1580

att gtc gat gcc ttt gtt gaa gaa gtc aag gtc ttc gag gag gac ttt   4800
Ile Val Asp Ala Phe Val Glu Glu Val Lys Val Phe Glu Glu Asp Phe
1585                1590                1595                1600

cgc aac agg aac cac atg cgc aaa ccc atc ttt acc gag acg cag tac   4848
Arg Asn Arg Asn His Met Arg Lys Pro Ile Phe Thr Glu Thr Gln Tyr
                1605                1610                1615

cgt gag atg gca atc cgg tgg acg cgg tcg tta gac gcg atg cgc gcg   4896
Arg Glu Met Ala Ile Arg Trp Thr Arg Ser Leu Asp Ala Met Arg Ala
            1620                1625                1630

atc ccg gat atc aac cag gat aaa gta gat cgg tat ggt gcc aaa ttc   4944
Ile Pro Asp Ile Asn Gln Asp Lys Val Asp Arg Tyr Gly Ala Lys Phe
        1635                1640                1645

atc cca ctt gtg gag cgg ttc tgg ggg aat tat cag gag atg atg ggg   4992
Ile Pro Leu Val Glu Arg Phe Trp Gly Asn Tyr Gln Glu Met Met Gly
    1650                1655                1660

gga ggg tat gat aat cct gct gtg gct ggc gat gag gat gat gat gag   5040
Gly Gly Tyr Asp Asn Pro Ala Val Ala Gly Asp Glu Asp Asp Asp Glu
1665                1670                1675                1680

ggc ccc agg agg aca gga aat gga aaa ggg ggg aat aag aag gga gga   5088
Gly Pro Arg Arg Thr Gly Asn Gly Lys Gly Gly Asn Lys Lys Gly Gly
                1685                1690                1695
```

```
gga gga gga gga gga aat gaa gta gtg gat ttg att agt agt gat gag   5136
Gly Gly Gly Gly Gly Asn Glu Val Val Asp Leu Ile Ser Ser Asp Glu
          1700                1705                1710

gat gaa cct ccg gct cgt gca cca tcg cgg aat gcg ggg cga gga aag   5184
Asp Glu Pro Pro Ala Arg Ala Pro Ser Arg Asn Ala Gly Arg Gly Lys
       1715                1720                1725

gca cag tcg aca cgt ggg gga caa atc caa gat aaa ggc cga gca gtc   5232
Ala Gln Ser Thr Arg Gly Gly Gln Ile Gln Asp Lys Gly Arg Ala Val
   1730                1735                1740

aac cgc cgc gga gaa ccc atc gcc gaa gaa gac gaa gaa gac tac ggg   5280
Asn Arg Arg Gly Glu Pro Ile Ala Glu Glu Asp Glu Glu Asp Tyr Gly
1745                1750                1755                1760

cta agc gac ccc gat atc gac gcc atc gat cca gac gcc atc acc gcc   5328
Leu Ser Asp Pro Asp Ile Asp Ala Ile Asp Pro Asp Ala Ile Thr Ala
               1765                1770                1775

tcc gac aac tcc gac gaa gaa gat gat gat gat gat gac gaa gac ctc   5376
Ser Asp Asn Ser Asp Glu Glu Asp Asp Asp Asp Asp Asp Glu Asp Leu
          1780                1785                1790

gaa tcc tcc cgc tac ttc tcc ggc tca aca ggc ccg ccc gtc tcc aaa   5424
Glu Ser Ser Arg Tyr Phe Ser Gly Ser Thr Gly Pro Pro Val Ser Lys
       1795                1800                1805

gcc gtg cag gat gct cga ctc cgt gaa caa ctt tcc atg tac gcc tcc   5472
Ala Val Gln Asp Ala Arg Leu Arg Glu Gln Leu Ser Met Tyr Ala Ser
   1810                1815                1820

ggc ggc agc tct tcg aaa ggt agc tac ggc tca ggg cgc gca tca gga   5520
Gly Gly Ser Ser Ser Lys Gly Ser Tyr Gly Ser Gly Arg Ala Ser Gly
1825                1830                1835                1840

gga tct tcg tcg aga gcg tcg gga tca gga tgg aga ggt gga gga gca   5568
Gly Ser Ser Ser Arg Ala Ser Gly Ser Gly Trp Arg Gly Gly Gly Ala
               1845                1850                1855

ggt ggg aag aaa tac tac agg aag aag agg gct ggt tct tcg gct gct   5616
Gly Gly Lys Lys Tyr Tyr Arg Lys Lys Arg Ala Gly Ser Ser Ala Ala
          1860                1865                1870

ggt ggt ggt ggt gca gga gga ggg gga gtt aca aaa cgg aag gcg agt   5664
Gly Gly Gly Gly Ala Gly Gly Gly Gly Val Thr Lys Arg Lys Ala Ser
       1875                1880                1885
```

```
ggg agt ggc gcg aag acg gcg agg aag agg ggt gca tct act gcg ccg   5712
Gly Ser Gly Ala Lys Thr Ala Arg Lys Arg Gly Ala Ser Thr Ala Pro
   1890                1895                1900

aag aca acg acg aga ggg gga gga tct gga gct ggg tct aga gga ggc   5760
Lys Thr Thr Thr Arg Gly Gly Gly Ser Gly Ala Gly Ser Arg Gly Gly
1905                1910                1915                1920

ggt gct ggt ggt gct ggt ggt gct ggt gct ggt gct ggt gct gga gga   5808
Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly Ala Gly Ala Gly Gly
            1925                1930                1935

ggg aaa agg ggt ggt gga ggt gga gga gga atg gga ggg ata agt gtt   5856
Gly Lys Arg Gly Gly Gly Gly Gly Gly Gly Met Gly Gly Ile Ser Val
          1940                1945                1950

atg cct cat tag                                                  5868
Met Pro His
      1955
```

<210> 3
<211> 1955
<212> PRT
<213> Neurospora crassa

<400> 3

```
Met Ala Lys Leu Ser Val Lys Asn Asn Leu Pro Arg Pro His Leu Val
  1               5                  10                  15
Ser Leu Ser Ser Ser Thr Thr Gly Ser Gly Ser Gly Ser Ala Ser Arg
             20                  25                  30
Ser Ala Ser Ala Lys His Gly Ser Ala Gly Ser Ser Thr Phe Asp His
         35                  40                  45
Glu Gln His Gln Gln His Gln Gln Gln Gln Gln Gln Lys Arg Gln Arg
     50                  55                  60
Ser Gln Ser Glu Ala Arg Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
 65                  70                  75                  80
Gln Gln Gln Gln Gln Gln Gln Gln Ala Gln His His Ala His Ser Thr
                85                  90                  95
Tyr Ala Gln Arg Pro Gln Pro Thr Pro Gln Gln Arg Pro Pro Gln Asn
            100                 105                 110
Leu Leu Thr Pro Ala Ser Thr Thr Gly Ala Ser Val Gly Pro Leu Gln
        115                 120                 125
Arg Ala Tyr Ser Val Ser Leu Ala Ala Arg Gln Ser Pro Ser Thr Asn
    130                 135                 140
Leu Val Arg Pro Lys Thr Asp Ser Pro Ala Pro His Thr Leu His Leu
145                 150                 155                 160
Lys Asn Lys Lys Asn Leu Arg His Pro Ala Pro Thr Pro Asp Ser Pro
```

```
                165                 170                 175
Ile Val Asp Asp Asp Ile Phe Ser Asp Ala Val Asp Leu Thr Glu Glu
            180                 185                 190
Leu Asp His Asp His Asp Leu Asn Gly Lys Asp Lys Asp Asn Thr Asp
        195                 200                 205
Asn Asp Asn Thr Val Ala Ser Ser Ser Leu Ile Gly Phe Gly Asp Asp
    210                 215                 220
Lys Leu Leu Trp Arg Glu Asp Phe Ala Glu Arg Ala Glu Pro Glu His
225                 230                 235                 240
Glu Arg Gly Gly Ser Arg Pro Arg Gln Val Lys Lys Arg Lys Ile Ser
                245                 250                 255
Asn Asp Tyr Ile Met Lys Asp Glu Asp Val Ser Leu Phe Asp Asp Asp
            260                 265                 270
Gly Glu Glu Asp Glu Phe Met Asp Ile Asn Glu Leu Val Gln Gly Asp
        275                 280                 285
Arg Glu Ser Thr Pro Lys Pro Lys Ala Thr Ser Arg Ser Val Ser Thr
    290                 295                 300
Arg Leu Pro Pro Thr Val Ser Leu Gln Arg Gly Arg Ser Pro Lys Arg
305                 310                 315                 320
Lys Glu Ala Ser Val Glu Lys Arg Thr Thr Glu Asn Gln Gln Gln Ala
                325                 330                 335
Asp Arg Glu Asp Glu Pro Ser Phe Met Ser Ser Pro Asp Val Asp Asn
            340                 345                 350
Ser Arg Lys Arg Lys Ser Ser Gly Ser Pro Thr Gly Leu Thr Thr Pro
        355                 360                 365
Arg Pro Gln Gln Lys Gln Thr Glu Glu Val Pro Gly Thr Thr Thr Ala
    370                 375                 380
Lys Lys Pro Arg Arg Ser Glu Val Met Asp Ser Glu Asp Glu Ala Phe
385                 390                 395                 400
Thr Pro Leu Ser Ala Gly Ser Leu Pro Gly Ser Ala Glu Phe Phe Arg
                405                 410                 415
Ser Gly Gly Thr Thr Thr Arg Glu Leu Gly Leu Asp Glu Asp Thr Val
            420                 425                 430
Met Asp Thr Pro Ser Arg Pro Pro Val Glu Ser Thr Leu Pro Thr Leu
        435                 440                 445
Glu Ser Val Glu Ser Arg Pro Pro Pro Leu Pro Pro Met Asp Leu Pro
    450                 455                 460
Ser Gln Arg Lys Pro Leu Glu Pro Leu Asn Thr Pro Arg Asn Gln Leu
465                 470                 475                 480
Leu Glu Ser Val Glu Arg Pro Thr Gln Gln Pro Ser Val Gly Pro Ser
                485                 490                 495
Phe Ala Gln Ser Ser Thr Leu Ala Glu Ser Ser Leu Pro Pro Ser Met
            500                 505                 510
Pro Pro Pro Ser Glu Asp Pro Leu Asn Thr Arg Glu Asn Ser Asn Leu
        515                 520                 525
Glu Glu Phe Asp Tyr Lys Leu Tyr Lys Pro Leu Leu Asp Leu Phe Val
    530                 535                 540
Asn Ala Pro Ala Ile Leu Glu Arg Glu Leu Ser Ala Val Asn Asp Glu
```

545 550 555 560
Leu Gln Glu Asn Met Ile Lys Leu Arg Asp Cys Leu Arg Leu Pro Arg
565 570 575
Glu Glu Arg Asp Arg Ala Arg Glu Glu Val Lys Lys Glu Lys Glu Met
580 585 590
Leu Lys Arg Arg Asp Ile Ala Leu Arg Ala Leu Gln Asp Glu His Lys
595 600 605
Leu Tyr Val Lys Lys Arg Lys Glu His Asn Leu Ile Asn Glu Glu Ile
610 615 620
Val Arg Ala Tyr Ala Glu Glu Asp Asp Glu Tyr Glu Asp Gln Leu Met
625 630 635 640
Ala Gln Leu Asp Lys Leu Asp Asp Glu Val Glu Ala Ile Val Lys Ser
645 650 655
Leu Thr Arg Leu Ile Val Ala Ala Gly Ile Thr Glu Lys Ser Phe Asp
660 665 670
Leu Lys Lys Glu Glu Glu Glu Glu Glu Glu Lys Pro Ile Ile Ile Ala
675 680 685
Thr Pro Thr Pro Ser Thr Arg Thr Glu Ala Pro Val Leu Pro Thr Thr
690 695 700
Glu Tyr His Asn Ser Gln Gln Val Ile Leu Gln Thr Gln His Pro Ala
705 710 715 720
Ala Gln Gln Val Ser His Arg Val Pro Pro Pro Pro Thr Pro Ser Phe
725 730 735
Gln Thr Ala Arg Gln Thr Pro Val Ser Tyr Gln Ser Arg Pro Thr Asn
740 745 750
Asn Ser Phe Pro Asp Ile Ser Ala Glu Glu Ala Met Met Phe Asp Lys
755 760 765
Glu Asp Pro Phe Met Glu Gln Gln His Ala Pro Ala Ser Ala Pro Phe
770 775 780
Gln Ala Thr Leu Pro Gln Arg Asn Ser Pro Phe Lys Thr Ala Pro Phe
785 790 795 800
Lys Pro Val His Gly His Asp Tyr Phe Asp Asp Glu Asp Asp Asp Ala
805 810 815
Asp Leu Leu Ala Ala Val Asp Ser Ala Glu Thr Tyr Thr Ser Thr Ala
820 825 830
Ala Thr Thr Thr Thr Asn Asn Asn Asn His Leu Arg Ser Gln Ser Val
835 840 845
Met Ser Thr Ser Thr Ala Thr Thr Ile Lys Pro Arg Lys Arg Asn Glu
850 855 860
Asn Ala Asn Ala Lys Lys Pro Lys Ser Val His Ala Lys Leu Ser Met
865 870 875 880
Pro Pro Glu Lys Met Lys Tyr Ala Trp Ser Asn Asp Val Arg Lys Ala
885 890 895
Leu Lys Asp Arg Phe Arg Met Ser Gly Phe Arg Gln Asn Gln Leu Glu
900 905 910
Ala Ile Asn Ala Thr Leu Gly Gly Lys Asp Ala Phe Val Leu Met Pro
915 920 925
Thr Gly Gly Gly Lys Ser Leu Cys Tyr Gln Leu Pro Ala Val Val Arg

930 935 940

Ser Gly Lys Thr Arg Gly Ile Thr Val Val Ile Ser Pro Leu Leu Ser
945 950 955 960

Leu Met Leu Asp Gln Val Asn His Leu Ala Asn Leu Met Ile Gln Ala
965 970 975

Tyr Ala Phe Asn Gly Asp Met Asn Ser Glu Met Arg Arg Met Val Phe
980 985 990

Gln Lys Leu Asp Ala Glu His Pro Glu His Glu Leu Gln Leu Leu Tyr
995 1000 1005

Val Thr Pro Glu Met Val Ser Lys Asn Gln Thr Phe Val Asn Lys Met
1010 1015 1020

Met Asp Leu Tyr Arg Arg Lys Lys Leu Ala Arg Ile Val Ile Asp Glu
1025 1030 1035 1040

Ala His Cys Val Ser Gln Trp Gly His Asp Phe Arg Pro Asp Tyr Lys
1045 1050 1055

Ala Ile Gly Glu Phe Arg Lys Arg Phe Pro Gly Val Pro Val Met Ala
1060 1065 1070

Leu Thr Ala Thr Ala Thr Gln Asn Val Ile Leu Asp Val Lys His Asn
1075 1080 1085

Leu Ala Met Glu Asp Cys Gln Thr Phe Ser Gln Ser Phe Asn Arg Pro
1090 1095 1100

Asn Leu Tyr Tyr Glu Val Arg Met Lys Glu Gln Asn Leu Ile Ala Arg
1105 1110 1115 1120

Ile Ala Glu Leu Ile Lys Glu Lys Tyr Asp Gly Gln Thr Gly Ile Ile
1125 1130 1135

Tyr Thr Leu Ser Arg Lys Ser Ala Glu Asn Ile Ala Lys Asn Leu Gln
1140 1145 1150

Glu Lys His Arg Ile Lys Ala Lys His Tyr His Ala Ser Ile Thr Thr
1155 1160 1165

Asp Glu Lys Ile Ser Val Gln His Glu Trp Gln Thr Gly Arg Val Lys
1170 1175 1180

Val Val Val Ala Thr Ile Ala Phe Gly Met Gly Ile Asp Lys Pro Asp
1185 1190 1195 1200

Val Arg Phe Val Ile His Gln His Ile Pro Lys Ser Leu Glu Gly Tyr
1205 1210 1215

Tyr Gln Glu Thr Gly Arg Ala Gly Arg Asp Gly Lys Pro Ser Asp Cys
1220 1225 1230

Tyr Leu Tyr Phe Ala Tyr Gly Asp Ile Gln Ser Leu Arg Arg Met Ile
1235 1240 1245

Ala Asp Gly Glu Gly Asp Tyr Ala Gln Lys Glu Arg Gln Leu Gln Met
1250 1255 1260

Leu Asn Arg Val Val Ser Tyr Cys Glu Ser Gln His Thr Cys Arg Arg
1265 1270 1275 1280

Glu Glu Val Leu Arg Tyr Phe Gly Glu Glu Phe Asp Tyr Arg Lys Cys
1285 1290 1295

Arg Asp Gly Cys Asp Asn Cys Arg Asn Gly Arg Ile Ser Lys Ser Thr
1300 1305 1310

Glu Met Arg Asp Phe Thr Glu Ile Ala Phe Ala Ala Ile Glu Val Val

1315 1320 1325

Lys Ser Gln Gln Pro Ile Thr Leu Gly Lys Leu Cys Asp Ile Leu Met
1330 1335 1340

Gly Lys Arg Lys Asn Glu His Gly Gly Val Cys His Phe Gly Ile Ala
1345 1350 1355 1360

Lys Gly Ser Thr Gln Arg Glu Leu Gln Arg Ile Val Leu Gln Leu Asn
1365 1370 1375

Phe His Lys Ala Leu Gly Glu Asp Asn Ile Met Asn Gly Ala Gly Met
1380 1385 1390

Pro Ile Thr Tyr Tyr Ile Thr Gly Pro Glu Ala Gly Ala Tyr Leu Tyr
1395 1400 1405

Asn Gly Lys Arg Leu Met Leu Pro Val Pro Ser Asn Lys Ser Val Glu
1410 1415 1420

Pro Pro Ser Arg Ser Lys Gln Arg Ser Arg Arg Val Asp Glu Asp Met
1425 1430 1435 1440

Asp Glu Gln Glu Leu Ser Thr Leu Gln Arg Pro Pro Thr Ser Thr Asn
1445 1450 1455

Val Ser Ser Pro Val Arg Ala Thr Lys Lys Arg Ser Ser Lys Lys Ala
1460 1465 1470

Leu Pro Thr Leu Ile Ala Asp Tyr Glu Glu Pro Ser Ser Asp Gly Pro
1475 1480 1485

His Gly Pro Leu His Ala Asn Gly Tyr Glu Arg Asp Asn Phe Val Val
1490 1495 1500

Ser Asp Asn Val Glu Pro Glu Glu Glu Glu Asp Ala Phe Glu Pro Val
1505 1510 1515 1520

Arg Pro Ser Arg Arg Gly Pro Ser Ser Arg Ala Thr Arg Pro Gln His
1525 1530 1535

Arg Gln Thr Thr Leu Tyr Asp Thr Leu Ser His Thr Gln Gln Ser Gln
1540 1545 1550

Thr Val Ser Gln His Leu Ala Thr Leu Gly Pro Pro Ile Asp Ala Arg
1555 1560 1565

Thr Met His Asn Pro Arg Tyr Ala Gln Leu Asp Glu Val His Gln Asp
1570 1575 1580

Ile Val Asp Ala Phe Val Glu Glu Val Lys Val Phe Glu Glu Asp Phe
1585 1590 1595 1600

Arg Asn Arg Asn His Met Arg Lys Pro Ile Phe Thr Glu Thr Gln Tyr
1605 1610 1615

Arg Glu Met Ala Ile Arg Trp Thr Arg Ser Leu Asp Ala Met Arg Ala
1620 1625 1630

Ile Pro Asp Ile Asn Gln Asp Lys Val Asp Arg Tyr Gly Ala Lys Phe
1635 1640 1645

Ile Pro Leu Val Glu Arg Phe Trp Gly Asn Tyr Gln Glu Met Met Gly
1650 1655 1660

Gly Gly Tyr Asp Asn Pro Ala Val Ala Gly Asp Glu Asp Asp Asp Glu
1665 1670 1675 1680

Gly Pro Arg Arg Thr Gly Asn Gly Lys Gly Gly Asn Lys Lys Gly Gly
1685 1690 1695

Gly Gly Gly Gly Gly Asn Glu Val Val Asp Leu Ile Ser Ser Asp Glu

1700 1705 1710

Asp Glu Pro Pro Ala Arg Ala Pro Ser Arg Asn Ala Gly Arg Gly Lys

1715 1720 1725

Ala Gln Ser Thr Arg Gly Gly Gln Ile Gln Asp Lys Gly Arg Ala Val

1730 1735 1740

Asn Arg Arg Gly Glu Pro Ile Ala Glu Glu Asp Glu Glu Asp Tyr Gly

1745 1750 1755 1760

Leu Ser Asp Pro Asp Ile Asp Ala Ile Asp Pro Asp Ala Ile Thr Ala

1765 1770 1775

Ser Asp Asn Ser Asp Glu Glu Asp Asp Asp Asp Asp Asp Glu Asp Leu

1780 1785 1790

Glu Ser Ser Arg Tyr Phe Ser Gly Ser Thr Gly Pro Pro Val Ser Lys

1795 1800 1805

Ala Val Gln Asp Ala Arg Leu Arg Glu Gln Leu Ser Met Tyr Ala Ser

1810 1815 1820

Gly Gly Ser Ser Ser Lys Gly Ser Tyr Gly Ser Gly Arg Ala Ser Gly

1825 1830 1835 1840

Gly Ser Ser Ser Arg Ala Ser Gly Ser Gly Trp Arg Gly Gly Gly Ala

1845 1850 1855

Gly Gly Lys Lys Tyr Tyr Arg Lys Lys Arg Ala Gly Ser Ser Ala Ala

1860 1865 1870

Gly Gly Gly Gly Ala Gly Gly Gly Gly Val Thr Lys Arg Lys Ala Ser

1875 1880 1885

Gly Ser Gly Ala Lys Thr Ala Arg Lys Arg Gly Ala Ser Thr Ala Pro

1890 1895 1900

Lys Thr Thr Thr Arg Gly Gly Gly Ser Gly Ala Gly Ser Arg Gly Gly

1905 1910 1915 1920

Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly Ala Gly Ala Gly Gly

1925 1930 1935

Gly Lys Arg Gly Gly Gly Gly Gly Gly Gly Met Gly Gly Ile Ser Val

1940 1945 1950

Met Pro His

1955

PCT/IT 99/00391

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C12N15/63 C12N15/67 C12N15/70 C12N15/74
C12N15/80 C12N15/82 C12N15/85 C12N15/11 C12N9/90
C12N1/19 C12N1/21 C12N5/10 C07K14/37 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 17979 A (NEW YORK BLOOD CENTER INC) 22 May 1997 (1997-05-22) 47.2% identity in 439 aa overlap with amino acids 897-1330 of SeqIdNo.3	1,2,7, 10,11, 16,18,19
X	-& DATABASE GENESEQ EBI, Hinxton, U.K. Accession Number: W31551, 27 January 1998 (1998-01-27) ELLIS N ET AL: "Bloom's syndrome BLM mutated protein" XP002136373 47.2% identity in 439 aa overlap with amino acids 897-1330 of SeqIdNo.3 abstract	1,2,18, 19

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 April 2000	11/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Lonnoy, O

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	COGONI C ET AL: "Isolation of quelling-defective (qde) mutants impaired in posttranscriptional transgene-induced gene silencing in Neurospora crassa" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 94, no. 19, 16 September 1997 (1997-09-16), pages 10233-10238, XP002136370 cited in the application table 2	1-23
Y	LINDEN H ET AL: "White collar 2, a partner in blue-light signal transduction, controlling expression of light-regulated genes in Neurospora crassa" EMBO JOURNAL., vol. 16, no. 1, 2 January 1997 (1997-01-02), pages 98-109, XP002136371 page 99, column 1, paragraph 3 -page 100, column 1, paragraph 2	1-23
A	SHERMAN J M ET AL: "An uncertain silence" TRENDS IN GENETICS,NL,ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 13, no. 8, 1 August 1997 (1997-08-01), pages 308-313, XP004084604 ISSN: 0168-9525	
A	COGONI C ET AL: "Quelling: transgene-induced gene silencing in Neurospora crassa" NATO ADVANCED STUDY INSTITUTE SERIES, SERIES H CELL BIOLOGY, vol. 104, 1998, pages 103-112, XP000906708	
T	COGONI C AND MACINO G: "Posttranscriptional gene silencing in Neurospora by a RecQ DNA helicase" SCIENCE., vol. 286, no. 5448, 17 December 1999 (1999-12-17), pages 2342-2344, XP002136372 the whole document	1-24

Information on patent family members

International Application No
PCT/IT 99/00391

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9717979 A	22-05-1997	US 5824501 A	20-10-1998
		CA 2237356 A	22-05-1997
		EP 0862442 A	09-09-1998